ABSTRACT

Aim: As diabetes mellitus and its complications become more prevalent in the world, it is becoming an important public health problem. Previous studies have investigated vitamin D in the context of diabetes mellitus and its complications. Microalbuminuria is important as the initial level of diabetic nephropathy. In this context, we aimed to investigate the level and deficiency of vitamin D in diabetic patients with microalbuminuria.

Material and Methods: 52 type 1 (20.1%) and 206 (79.9%) type 2 diabetes patients who applied to the outpatient Endocrinology and Metabolic Diseases clinic between April 2019 and December 2020 were included in the study. Patients were classified according to microalbuminuria. Fasting glucose, HbA1c, duration of diabetes, and 25 (OH) Vitamin D levels and mean waist circumference were compared between the groups. Finally, the groups were compared according to diabetes type and microalbuminuria.

Results: Urinary albumin/creatinine rates (UAC) in 159 (42.7%) diabetic patients were in normal range, and 65 (17.5%) diabetics had UAC between 30-300 mcg. 12 (3.2%) had UAC >300 mcg. 22 (5.9%) had chronic renal failure. Vitamin D deficiency was 61.6% and vitamin D insufficiency was 28.6% in all study groups. Median GFR was 98 (38-136) ml/dk and median 25 (OH) D level was 17.1 (5.0-44.2) mg/dl. 25 (OH) Vitamin D levels and GFR were found to be significantly lower in the microalbuminuria group (p<0.01). 25 (OH) D levels were found to be low in both type 1 and type 2 diabetes patients with the complication of microalbuminuria, however only in type 1 diabetes patients low vitamin D this was found significant (p=0.01)

Conclusion: 25 (OH) vitamin D deficiency and insufficiency were found to be more common in patients with diabetes with microalbuminuria, which was more significant in type 1 diabetes patients The underlying mechanisms and potential therapeutic effect of vitamin D should be further investigated.

Keywords: Diabetes, Microalbuminuria, Vitamin D

Diabetes Mellituslu Hastalarda Mikroalbuminüri ve D Vitamini

ÖZ

Amaç: Dünyada giderek artan sküllüğü ile diyabet, komplikasyonları yolu ile önemli bir halk sağlığı sorunu olarak karşımıza çıkmaktadır. Önceki çalışmalar vitamin D için hem diyabet hem de komplikasyonları ile ilişkili olarak değerlendirilmiştir. Mikroalbuminüri diyetetik nefropatinin başlangıç düzeyi olarak önem arıtmadır. Bu bağlamda mikroalbuminüriyi olan diyabetik hastalarda D vitamini düzeyini ve eksikliği araştırmayı amaçladık.

Gereç ve Yöntemler: Çalışmaya Nisan 2019 ve Aralık 2020 tarihleri arasında Endokrinoloji ve Metabolizma Hastalıkları poliklininine başvuran 52 tip 1 (%20,1) ve 206 (%79,9) tip 2 diyetet hastası dahil edildi. Hastalar mikroalbuminüriyi gören 25 (OH) vitamin D düzeyleri ve ortalamalar bel çevresi grupler arasında karşılaştırıldı. Son olarak gruplerin diyabet tipi ve mikroalbuminüriyi göre karşılaştırıldı.

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**INTRODUCTION**

Diabetic nephropathy (DN) is a long-term microvascular complication of both type 1 and type 2 diabetes that can progress to end stage renal disease and is one of the most important complications leading to higher morbidity and mortality. DN can be detected early by urinary microalbumin as recommended by the American Diabetes Association (ADA) (1).

Microalbuminuria is not only an important sign of diabetic nephropathy, but also contributes to cardiovascular mortality in diabetes (2,3). Its frequency in type 1 diabetic patients was 15% and in type 2 diabetic patients ranged from 25.6-29.7% according to previous studies (4-6).

In recent years, there have been significant advances in the understanding of 25 (OH) vitamin D and its effects beyond its known role in bone and mineral metabolism. Preliminary studies demonstrated 25 (OH) vitamin D deficiency in the pathogenesis of diabetes (7). In addition, vitamin D supplementation has been shown to increase insulin sensitivity and secretion (8,9).

There are some studies that have investigated the effect of vitamin D on glomerular function. Vitamin D decreased podocyte loss and albuminuria in rat models, with active Vitamin D treatment fewer signs of podocyte injury and podocyte hypertrophy reversal were observed in subtotally nephrectomized rats (10). In animal studies, active Vitamin D slowed down mesangial proliferation and negatively affected RAAS activation and Vitamin D receptor null mice were shown to have several fold elevated renin expression and angiotensin 2 production (11).

Therefore, we aimed to investigate the association between 25 (OH) vitamin D levels and microalbuminuria in patients with type 1 and type 2 diabetes mellitus. Our study was unique that it compared relationship between 25(OH) Vitamin D deficiency and microalbuminuria according to types of diabetes.

**MATERIALS and METHODS**

52 type 1 (20.1%) and 206 (79.9%) type 2 diabetes patients, aged between 18-70 patients were enrolled in the study. All patients were regularly followed up by Endocrinology and Metabolism department between April 2019 and December 2020. Age smaller than <18, pregnancy, liver disease, active infection, use of any medications, or any illness that affects 25(OH) vitamin d levels were exclusion criteria. Weight (kg) and height (m) were measured using standard methods. The waist circumference (WC) was measured at the midpoint between the underside of the chest and the uppermost edge of the iliac crest in the standing position.

All the laboratory analyses were studied in the same laboratory. Serum creatinine (Cre) was measured by enzyme-linked immunosorbent assay (ELISA) and glomerular filtration rate (GFR) was measured by CKD- EPI method (ml/dk/1,75m²) Urine microalbuminuria was measured by immunoturbidometric method. Microalbuminuria was defined as urine albumin 30-300 mg/g, macroalbuminuria was defined as urine albumin > 300mg/g (12). 25 (OH) Vitamin D was measured by high-performance liquid chromatography. Patients were divided into type 1 and type 2 according to previous records. Vitamin D deficiency was defined as 25 (OH) Vitamin D <20 ng/ml, Vitamin D insufficiency was defined as 25 (OH) Vitamin D between.21-29 ng/ml (13).

Ethical approval was taken from Istanbul Research and Educational Hospital (2021/2784).

**Statistics**

Statistical evaluations were performed using IBM SPSS 22.0 (Statistical Package for the Social Sciences software version 22.0). Descriptive analyses were expressed as median (min-max) or mean±standard deviation (SD) and percentages (%). Shapiro-Wilk test was used for normality. The Chi-square test or Fisher’s exact test, where appropriate, was used for categorical variables. Student’s t-test was
results
Totally 258 diabetes patients were included in the study. The median age was 54 years (min 21-max 70). 50 patients were type 1 diabetes patients, 208 patients were type 2 diabetes patients, mean glucose level was 198 ±73.75, mean body mass index was 32.16±6.74 kg/m². Median urine albumin/creatinine level was 14.0 mg/g (1.3-633). The general physical and biochemical properties of the groups were given in Table 1.

159 (42.7%) patients had normal urine albumine/creatinine ratio (<30 mcg) (UAC). 65 (17.5%) patients had UAC between 30-300mcg. 12 (3.2%) had UAC >300 mcg. 22 (5.9%) had chronic renal failure. General characteristics according to microalbuminuria were presented in Table 2.

Vitamin D deficiency was found 61.6%, vitamin D insufficiency was found 28.6% in all study groups. GFR and mean 25 (OH) D level were found significantly lower in the microalbuminuria group. (p=0.01) (Table 2, 3).

25 (OH) D levels were lower in both type 1 and type 2 diabetes microalbuminuria patients. The p-value of 25 (OH) D in microalbuminuria patients was lower in type 1 diabetes patients.(p=0.01) (Table 4)

Table 1: General characteristics of the patients.

| Characteristics                  | Findings                   |
|----------------------------------|----------------------------|
| Age [years, median (min-max)]    | 54 (21-70)                 |
| Gender [Female / Male (%)]       | 48.1 / 51.9               |
| Diabetes Duration [years, median (min-max)] | 11 (1-30)           |
| HbA1c [%, median (min-max)]      | 8.8 (5.6-15.3)            |
| WC (cm±SS)                       | 104.6 ±16.4               |
| GFR [ml/min/1.73 m², median (min-max)] | 98 (38-136)          |
| 25 (OH) D [mg/dl, median (min-max)] | 17.1 (5.0-44.2) |

Median (min-max), mean±standard deviation for WC, WC: Waist circumference, GFR: Glomerular filtration rate.

Table 2: General characteristics according to microalbuminuria.

| Parameters                        | MAU<30 mg/g (n=155) | MAU>30 mg/g (n=98) | p    |
|-----------------------------------|---------------------|---------------------|------|
| Age [years, median (min-max)]     | 53.5 (21-70)        | 55 (24-67)          | 0.06 |
| Gender [Female, n (%)]            | 75 (48.4)           | 53 (54.1)           | 0.38 |
| Diabetes Duration [years, median (min-max)] | 10 (1-30)          | 12 (2-30)           | 0.68 |
| Glucose levels (mg/dl±SS)         | 215.2±102.6         | 207.3±90.6          | 0.55 |
| HbA1c [% , median (min-max)]      | 8.1 (5.6-15.3)      | 8.9 (5.6-14.7)      | 0.51 |
| WC (cm±SS)                        | 105.0±15.3          | 110.5 ±15.9         | 0.02 |
| Creatinine (mg/dl±SS)            | 0.7 (0.4-1.2)       | 0.9 (0.5-1.97)      | <0.01|
| GFR [ml/min/1.73 m², median (min-max)] | 100 (59-137)       | 87 (32-117)         | 0.01 |
| 25(OH) D [mg/dl, median (min-max)] | 20.0 (8.4-44.0)    | 15.2 (7.0-29.0)     | 0.01 |

Median (min-max), mean±standard deviation for Glucose and WC, Mann-Whitney U Test. Chi-Square test for gender, student’s t test for Glucose and WC, MAU: Microalbumin, WC: Waist circumference, GFR: Glomerular Filtration Rate, 25 (OH) D: 25 (OH) Vitamin, D, significant p values were given as bold.

Table 3: Status of vitamin D levels according to microalbuminuria.

| Status of Vitamin D Levels       | Cases with MAU >30 mg/g, n (%) | Cases with MAU>300 mg/g, n (%) |
|----------------------------------|--------------------------------|--------------------------------|
| Normal 25(OH) D (n=20)           | 5 (25)                         | 0 (0)                          |
| 25(OH) D Insufficiency (n=59)    | 16 (27.11)                     | 1 (1.6)                        |
| 25(OH) D Deficiency (n=127)     | 57 (45.2)                      | 10 (7.9)                       |
| p                                | 0.027                          | 0.084                          |

Chi-square test, MAU: Microalbumin/creatinine, significant p values were given as bold.
DISCUSSION
The results of the current study showed that microalbuminuria was more frequent in patients with 25 (OH) Vitamin D deficiency and the mean 25(OH) Vitamin D levels were lower in the microalbuminuria group.

All study groups had a high proportion of vitamin D deficiency and/or insufficiency in the study (90.2%). This was consistent with other prevalence studies. In a study from Turkey with 4860 outpatients, 25 (OH) vitamin D deficiency and insufficiency were found in 91.1% (14). In another study with 209 adults from the Aegean region of Turkey, 88.7% were found to have 25 (OH) vitamin D deficiency and insufficiency (15).

The current study showed that Vitamin D deficiency and insufficiency were observed in higher HbA1c values. This could be explained by possible several mechanisms. Vitamin D has been found to regulate insulin secretion from pancreatic beta cells (16,17), also it has been demonstrated that vitamin D coordinates epigenetic, redox control, mitochondrial function which results in reduced oxidative stress and defends tissues from toxins (18,19). Additionally; Vitamin D and its analogs prevent beta-cell degeneration from immune attack by several mechanisms, which is crucial for type 1 diabetes mechanism (20,21).

Analysis of results of the current study showed that higher urine albumin/creatinine ratio is associated together with low 25 (OH) Vitamin D. Previously; Levine et al. demonstrated low 1.25 (OH) D with high albumin/creatinine ratio in patients with chronic renal failure (22). Low Vitamin D had been shown to be associated with high angiotensin II levels which were increased in DN patients (23). In another study; paricalcitol administration of CRF patients has resulted in decreased proteinuria (24). A study from Iran also has pointed that treatment with Vitamin D decreased proteinuria in 60 patients with type 2 diabetes (25).

Current study demonstrated that the association of vitamin D deficiency with microalbuminuria was higher in type 1 diabetes patients (p=0.01). Therefore Vitamin D’s effects on different mechanisms responsible for progression of diabetic complications according to diabetes type should be more extensively investigated.

Limitations of these studies were its cross-sectional design, it reflects the findings of single-center experience, however, The sample size was much larger than was previously used in similar studies that will add new data to the literature.

In conclusion, 25 (OH) Vitamin D deficiency and insufficiency were found to be higher in patients with diabetes and microalbuminuria. Vitamin D deficiency was more pronounced especially in type 1 diabetes patients with microalbuminuria. Underlying mechanisms and the potential therapeutic effect of Vitamin D should be investigated more thoroughly.

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Authorship Contributions
Concept: Savaş Karataş, Design: Savaş Karataş, Data Collection or Processing: Savaş Karataş, Yalçın Hacıoğlu, Analysis or Interpretation: Savaş Karataş, Şennur Köse, Literature Search: Savaş Karataş, Yalçın Hacıoğlu, Şennur Köse, Writing: Savaş Karataş, Yalçın Hacıoğlu, Şennur Köse.

Conflicts of Interest
The authors declare that they have no competing interest.

Financial Disclosure
The authors received no financial support for this study.

Ethical Approval
The study was conducted with the written approval of the Ethics Board of a research and education hospital (2021/2784).

Table 4: Results of groups according to diabetes type and microalbuminuria.

| Parameters                        | T1D with MAU (+) (n=12) | T1D with MAU (-) (n=30) | p* | T2D with MAU (+) (n=69) | T2D with MAU (-) (n=97) | p** |
|----------------------------------|-------------------------|-------------------------|----|-------------------------|-------------------------|----|
| Serum Creatinine [mg/dl, median (min-max)] | 1.0 (0.6-1.3) | 0.8 (0.5-1.3) | 0.8 | 0.7 (0.4-1.4) | 0.9 (0.5-1.97) | 0.03 |
| GFR [ml/min/1.73 m², median (min-max)]   | 89 (69-137) | 104 (54-128) | 0.7 | 87 (32-137) | 101 (39-137) | 0.01 |
| Diabetes Duration [years, median (min-max)] | 12 (1-34) | 14 (0.3-35) | 0.48 | 10 (1-30) | 11 (2-33) | 0.86 |
| WC (cm±SS)                        | 96.43±11.52 | 81.8±9.83 | **0.12** | 107.49±15.34 | 112.88±13.88 | **0.02** |
| 25 (OH) D [mg/dl, median (min-max)] | 10.0 (5.0-22.1) | 18.0 (9.1-44.2) | **0.01** | 16.0 (7.0-24.6) | 18.0 (8.4-30.0) | **0.09** |

Median (min-max), mean±standard deviation for WC, Mann Whitney U Test, student’s t test for WC Creatinine: Cre, MAU: Microalbumin, WC: Waist circumference, GFR: Glomerular Filtration Rate, 25 (OH) D: 25 (OH) Vitamin, D, significant p values were given as bold.

T1D with MAU (+): Type 1 Diabetes with Microalbuminuria
Peer Review Process

Extremely peer reviewed and accepted.

REFERENCES

1. American Diabetes Association. 11. Microvascular Complications and Foot Care: Standards of Medical Care in Diabetes—2021. Diabetes Care. 2021;44(Suppl 1):S151-S167.

2. Svensson MK, Cederholm J, Eliasson B, Zethelius B, Gudbjörnsdottir S; Swedish National Diabetes Register. Albuminuria and renal function as predictors of cardiovascular events and mortality in a general population of patients with type 2 diabetes: A nationwide observational study from the Swedish National Diabetes Register. Diab Vasc Dis Res. 2013;10(6):520-529.

3. Yuyun MF, Dinneen SF, Edwards OM, Wood E, Wareham NJ. Absolute level and rate of change of albuminuria over 1 year independently predict mortality and cardiovascular events in patients with diabetic nephropathy. Diabet Med. 2003;20(4):277-282.

4. Briet C, Piffaretti C, Fosse S, Denis P, Allix I, Campagna AF, Coutant R. Épidémiologie du diabète de type 1 et de ses complications [Epidemiology of type 1 diabetes and its complications]. Rev Prat. 2018;68(6):607-610.

5. Sana MA, Chaudhry M, Malik A, Iqbal N, Zakiuddin A, Abdullah M. Prevalence of microalbuminuria in type 2 diabetes mellitus. Cureus. 2020;12(12):e12318.

6. Asadujjaman M, Kashem A, Chowdhury AA, Roy AS, Muqueet MA, Fazilatunnasa M, Ahammed SU, Rabbani MG, Rahman MA, Kabir MS, Hosain MB, Islam MS, Das SK, Khan EH, Borman GC, Khatun N. Prevalence of microalbuminuria and overt proteinuria in diabetes mellitus and their association with renal function. Mymensingh Med J. 2018;27(3):467-474.

7. Wimalawansa SJ. Associations of vitamin D with insulin resistance, obesity, type 2 diabetes, and metabolic syndrome. J Steroid Biochem Mol Biol. 2018;175:177-189.

8. Gulseth HL, Wium C, Angel K, Eriksen EF, Birkeland KI. Effects of vitamin d supplementation on insulin sensitivity and insulin secretion in subjects with type 2 diabetes and vitamin d deficiency: A randomized controlled trial. Diabetes Care. 2017;40(7):872-878.

9. Nazarian S, St Peter JV, Boston RC, Jones SA, Mariah CN. Vitamin D3 supplementation improves insulin sensitivity in subjects with impaired fasting glucose. Transl Res. 2011;158(5):276-281.

10. Kuhlmann A, Haas CS, Gross ML, Reulbach U, Holzinger M, Schwarz U, Ritz E, Amann K. 1,25-Dihydroxyvitamin D3 decreases podocyte loss and podocyte hypertrophy in the subtotal nephrectomized rat. Am J Physiol Renal Physiol. 2004;286(3):F526-F533.

11. Li YC, Kong J, Wei M, Chen ZF, Liu SQ, Cao LP. 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. J Clin Invest. 2002;110(2):229-238.

12. Ruggenenti P, Fassi A, Iliev IA, Brusegan V, Rubis N, Gherardi G, Arnuldi F, Ganeva M, Ene-Iordache B, Gaspari F, Perna A, Bossi A, Trevisan R, Dodesini AR, Remuzzi G; Bergamo Nephrologic Diabetes Complications Trial (BENEDICT) Investigators. Preventing microalbuminuria in type 2 diabetes. N Engl J Med. 2004;351(19):1941-1951.

13. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM; Endocrine Society. Evaluation, treatment, and prevention of vitamin D deficiency: An Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2011;96(7):1911-1930.

14. Sezgin G, Ozturk G, Turkal R, Caykara B. Vitamin D levels of outpatients admitted to a university hospital in the Marmara Region of Turkey over 3 years. J Med Biochem. 2019;38(2):181-187.

15. Hekimsoy Z, Dinç G, Kafesçiler S, Onur E, Güvenç Y, Pala T, Güçlü F, Ozmen B. Vitamin D status among adults in the Aegean region of Turkey. BMC Public Health. 2010;10:782.

16. Johnson JA, Grande JP, Roche PC, Kumar R. Immunohistochemical localization of the 1,25(OH)2D3 receptor and calbindin D28k in human and rat pancreas. Am J Physiol. 1994;267(3 Pt 1):E356-360.

17. Altieri B, Grant WB, Della Casa S, Orio F, Pontecorvi A, Colao A, Sarno G, Muscioguri G. Vitamin D and pancreas: The role of sunshine vitamin in the pathogenesis of diabetes mellitus and pancreatic cancer. Crit Rev Food Sci Nutr. 2017;57(16):3472-3488.

18. Berridge MJ. Vitamin D cell signalling in health and disease. Biochem Biophys Res Commun. 2015;460(1):53-71.

19. George N, Kumar TR, Antony S, Jayanarayanan S, Paulose CS. Effect of vitamin D3 in reducing metabolic and oxidative stress in the liver of streptozotocin-induced diabetic rats. Br J Nutr. 2012;108(8):1410-1418.

20. Casteels K, Waer M, Bouillon R, Depovere J, Valckx D, Laureys AE, Van Halteren AG, Tysma OM, Mathieu C, Roep BO. 1,25-Dihydroxyvitamin D3 restores sensitivity to cyclophosphamide-induced apoptosis in non-obese diabetic (NOD) mice and protects against diabetes. Clin Exp Immunol. 1998;112(2):181-187.

21. van Halteren AG, Tysma OM, van Etten E, Mathieu C, Roep BO. 1-alpha,25-dihydroxyvitamin D3 or analogue treated dendritic cells modulate human autoreactive T cells via the selective induction of apoptosis. J Autoimmun. 2004;23(3):233-239.

22. Levin A, Bakris GL, Molitch M, Sullens M, Tion J, Williams LA, Andress DL. Prevalence of abnormal serum vitamin D, PTH, calcium, and phosphorus in patients with chronic kidney disease: Results of the study to evaluate early kidney disease. Kidney Int. 2007;71(1):31-38.

23. Forman JP, Williams JS, Fisher ND. Plasma 25-hydroxyvitamin D and regulation of the renin-angiotensin system in humans. Hypertension. 2010;55(5):1283-1288.

24. Agarwal R, Acharya M, Tian J, Hippensteel RL, Melnick JZ, Qiu P, Williams L, Battle D. Antiproteinuric effect of oral paricalcitol in chronic kidney disease. Kidney Int. 2005;68(6):2823-2828.

25. Momeni A, Mirhosseini M, Kabiri M, Kheiri S. Effect of vitamin D on proteinuria in type 2 diabetic patients. J Nephropathol. 2017;6(1):10-14.