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# Vitamin D Receptor Gene Polymorphisms and Vitamin D Levels in Egyptian Patients with Diabetic Nephropathy and Type 2 Diabetes Mellitusus

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## Abstract

*Aim:* Type 2 diabetes and diabetic nephropathy are associated with multiple genetic variables. This study aimed to investigate the role of the vitamin D receptor (VDR) BsmI gene polymorphism and the serum level of vitamin D in Egyptian patients with diabetic nephropathy and type 2 diabetes.

*Material and Methods:* This study involved 80 patients with type 2 diabetes without nephropathy (T2DM), 80 patients with diabetic nephropathy (DN) and 100 healthy individuals as controls. VDR BsmI gene polymorphisms were determined by PCR-RFLP, and vitamin D serum levels were determined by the electrochemiluminescence binding assay.

*Results:* The distribution frequencies of the Bb, bb and Bb+bb genotypes in patients with DN were significantly different from those in the controls ($p$<0.001) and in those with T2DM ($p$<0.001). In addition, the b allele frequency was significantly higher in the DN group than the healthy control (OR=6.3, 95% CI, 3.7-10.7, ($p$<0.001) and T2DM groups (OR=1.91, 95% CI 4.0-13.5, $p$<0.001). The patients with DN had significantly lower levels of serum vitamin D than both the healthy controls and T2DM patients ($p$<0.001). Using multivariate regression analysis, diabetes duration, HbA1C, vitamin D deficiency and BsmI genotype were found to be independent risk factors for DN ($p$=0.019, 0.036, 0.001, 0.035, respectively).

*Conclusion:* The VDR BsmI gene polymorphism and vitamin D deficiency are risk factors for type 2 diabetes and diabetic nephropathy.

***Key Words:*** *Diabetes – Diabetic nephropathy – Egyptian population – Vitamin D deficiency – VDR gene polymorphisms – Risk assessment.*

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## Introduction

**DIABETES** mellitus is a metabolic disease characterized by hyperglycaemia that results from abnormal insulin levels, abnormal function or both. Chronic hyperglycaemia associated with diabetes may lead to abnormal function in various organs, especially the kidneys, eyes, nerves and blood vessels [1].

In Egypt, the prevalence of type 2 diabetes is approximately 15.56% among adults between 20 and 79 years of age, with an associated annual mortality rate of 86,478. Furthermore, reports indicate that 43% of patients with diabetes and most patients with prediabetes in Egypt are likely undiagnosed. It is alarming that the diabetes prevalence in Egypt has increased rapidly within a relatively short period, from approximately 4.4 million in 2007 to 7.5 million in 2013. It is expected that this number will increase to 13.1 million by 2035 [2].

The prevalence of chronic diabetes complications ranges from 8.1% to 41.5% for retinopathy [3], 21% to 22% for albuminuria [3,4], 6.7% to 46.3% for nephropathy [3,5] and 21.9% to 60% for neuropathy [3,5].

***Abbreviations:***

| | |
|---|---|
| T2DM | : Type 2 diabetes. |
| DN | : Diabetic nephropathy. |
| HTN | : Hypertension. |
| VDR | : Vitamin D receptor. |
| A/C ratio | : Albumin/creatinine ratio. |
| MBP | : Mean blood pressure. |
| BMI | : Body mass index. |
| HBA1C | : Glycated haemoglobin. |

Diabetic nephropathy (DN) occurs in 20-40% of patients with diabetes and is the single leading cause of end-stage renal disease (ESRD). Persistent microalbuminuria with levels ranging from 30-299 mg/24 h provides an indication of the early stage of DN in type 1 diabetes and is a sign of nephropathy in patients with type 2 diabetes (T2D) [6].

T2D is related to genetic factors [7]. Several genes associated with T2D susceptibility have been investigated, especially genes associated with oxidative stress, growth factors, and bone mineral density [8]. The vitamin D endocrine system plays an important role in the development of DM. High levels of vitamin D can enhance pancreatic b-cell secretion functions and improve insulin resistance [9].

The vitamin D receptor (VDR) gene is located on human chromosome 12q12-q14. The genetic polymorphism involved in the development of T2DM may have multiple susceptibility SNPs [10]. Of these genes, the BsmI (rs1544410) polymorphism is within the gene intron.

Vitamin D deficiency has mainly been reported in patients with chronic kidney disease or in diabetic patients with normal renal function, but few studies have investigated vitamin D levels in patients with DN. In addition, the causes of vitamin D deficiency in patients with DN are still unclear [11].

Thus, the present study aimed to investigate vitamin D status and VDR BsmI gene polymorphisms associated with DN susceptibility in Egyptian patients with type 2 diabetes.

## Patients and Methods

The present study was conducted within the Clinical Pathology and Internal Medicine Departments, College of Medicine, Menoufia University, during the period from May 2015 to October 2016.

*Study subjects:*

This study was performed in 160 patients and 100 healthy individuals as controls. The patients were divided into 2 groups according to microalbumin level in the urine, namely, 80 type 2 diabetic patients with urinary albumin excretion less than 30mg/g of creatinine (mg/g Cr; group B) and 80 type 2 diabetic patients with microalbuminuria (30-300mg/g Cr; group C).

All patients were selected from the Internal Medicine Department, Menoufia University. Written consent was obtained from all subjects. Type 2 diabetic patients were diagnosed according to the American Diabetes Association (ADA) criteria and had a fasting plasma glucose (FPG) level ≥126mg/dL (7.0mmol/L) or a 2-h post-load glucose concentration ›_200mg/dL (11. 1mmol/L) during the OGTT (75g) [12]. Patients with obstructive renal disease, abnormal urinary sediment, urinary tract infection, a history of glomerulonephritis, ureteric stones, dilated renal pelvis, severely atrophied kidney(s), acute or severe chronic liver disease, heart failure, acute febrile illness, and haematologic diseases, as well as those who were pregnant or used a renal dialysis machine, were excluded from the study.

The history of all subjects was obtained, including age, gender, and duration of diabetes and hypertension. A clinical examination was performed, including body mass index (BMI, $kg/m^2$) and mean blood pressure (MBP). Laboratory tests, including fasting blood glucose (2-h post-prandial using the Hexokinase Method with SYNCHRONCX9 autoanalyzer, Beckman, Instrument,Inc,scientific, Instrument, Division, Ful-lerton,CA92634100USA), determination of HbA1c by quantitative colorimetric measurement of glycohemoglobin as a percent of total haemoglobin (STANBIO LABORATORY), lipid profile (total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol) using an enzymatic colorimetric method with the SYNCHRON CX9, and low-density lipoprotein cholesterol (LDL-C), were performed and calculated using the Friedewald equation. Blood urea and creatinine (SYNCHRON CX9) levels and estimated glomerular filtration rate (GFR) using the Cockroft and Gault Formula were also collected for subjects [13].

*Determination of the albumin/creatinine ratio:*

The albumin/creatinine (Alb/Creat) ratio was calculated after estimation using the Beckman microalbumin test kit on a Beckman Synchron CX9 autoanalyzer. Microalbumin in urine was determined using the rate nephelometric methodology. The microalbumin creatinine ratio was calculated using the following equation: microalbumin/urinary creatinine=microalbumin (mg/dl) / urinary creatinine (g/dl). The ranges of this ratio are as follows: 0-29, normoalbuminuria; 30-300, microalbuminuria; and >300, macroalbuminuria [14].

*Determination of serum 25-hydroxyvitamin D (25(OH)D) level*:

The serum was stored at –20ºC to measure the 25-hydroxyvitamin D (25(OH)D) level using a electrochemiluminescence binding assay with a cobas analyser. The cut-off levels for vitamin D status according to the National Institute of Health,

Food and Nutrition Board were as follows: 25(OH) D level <30 nmol/L indicates a deficiency, 30 to <50nmol/L indicates an insufficiency, ≥50 nmol/L is normal (sufficient) and >100nmol/L is toxic [15].

*PCR-RFLP:*

DNA was extracted using a spin-column technique kit for DNA extraction from humans using whole blood with EDTA (GeneJET Whole Blood Genomic DNA Purification Mini kit). The vitamin D receptor gene BsmI (rs1544410) polymorphism was identified using polymerase chain reaction-restriction fragment length polymorphism analysis (16,17). PCR amplification was achieved using the following primers (Biosearch technology):F: CAACCAAGACTACAAGTACCGCGTCAGTGA & R: AACCAGCGG GAAGAGGTCAAGGG, with a total volume of 25 µl using the following: 12.5 µl of master mix (Promega), 1.5 µl of each primer (0.5 µmol), 8.5 µl of template DNA (100-500ng), and 1.0 µl of sterile deionized water for a final volume of 25 µl. The thermal cycler profile (Gene amp, PCR system 2400 PERKIN ELMER, USA, version 2.11) included an initial denaturation step at 94°C for one minute, followed by 29 cycles at 94°C for 60sec, 65°C for 60sec, and 72°C for 1min, and a final extension of 5min at 72°C.

The PCR product was subjected to digestion by BsmI restriction enzymes (New England Biolabs, USA). The digestion mixture was incubated for 16h at 37°C, and the PCR products were electrophoresed onto a 2% agarose gel. Electrophoresis was performed at room temperature for 45min. Samples yielding 650-bp and 175-bp fragments were scored as bb, those with single 825-bp fragments were scored as BB, and those with 825-bp, 650-bp and 175-bp fragments were scored as Bb genotypes. For each gel used, a DNA marker (Low Rang DNA Marker, 100bp) was applied for fragment determination.

*Statistical analysis:*

The collected data were analysed using SPSS software (version 20.0). Descriptive statistics in the form of the mean±SD were used for parametric data. The differences between the variables were evaluated by the chi-square test, Kruskal-Wallis test and one-way analysis of variance according to the data. Student's *t*-test, ANOVA, Fisher's exact test and the Mann-Whitney test were used. The association between genotypes and disease was assessed by computing the odds ratio (OR) and 95% confidence interval. Regression analysis was used to estimate the relationships among variables. The significance level was set at 0.05 or less.

## Results

*Clinical characteristics of the study subjects:*

In the present study, there were no significant differences between the groups with respect to age and sex. In terms of clinical features, the DN patients had a significantly longer duration of diabetes and duration of hypertension than the T2DM patients ($p$<0.001), but there was no difference between the two groups with respect to mean blood pressure ($p$=0.763). Both the T2DM and DN groups had a significantly higher BMI than the healthy control group, and the DN group had a higher BMI than the T2DM group ($p$<0.001), as shown in Table (1).

*Routine laboratory investigations of the study subjects:*

Both the T2DM and DN groups had significantly higher levels of FBG, 2HPP and HbA1c% than the healthy control group, and the DN group had higher levels than the T2DM group ($p$<0.001), as shown in Table (2). Regarding the lipid profile, both the T2DM and DN groups had significantly higher levels of cholesterol, TG, and LDL and lower levels of HDL than the healthy control group ($p$<0.001). In addition, the DN patients had higher cholesterol and LDL levels than the T2DM group ($p$<0.001), but there was no difference in TG or HDL levels between the groups (Table 2). Regarding kidney function, there was a significant difference in creatinine between the T2DM and DN groups ($p$<0.001), but there was no difference between the control and T2DM groups ($p$=0.215). Regarding the eGFR, there was no difference between the control group and T2DM ($p$=0.527), but the DN group had a lower level than the T2DM group ($p$<0.001) (Table 3).

*Microalbumin of the study subjects:*

To assess microalbuminuria, we measured microalbumin in the urine, and its ratio to creatinine was calculated. This ratio was significantly different between all the groups ($p$<0.001), as shown in Table (3).

*Vitamin D levels in the groups:*

Vitamin D levels were significantly decreased in both the T2DM and DN groups compared with those in the control group. DN patients also had lower levels of vitamin D than T2DM patients ($p$<0.001) (Table 3).

VDR BsmI genotype and allele distributions and risk of type 2 diabetes and diabetic nephropathy (Fig. 1 and Table 4).

Table (1): Comparison between cases and control regarding clinical data.

| | The studied groups | | | Test ANOVA | *p*-value |
|---|---|---|---|---|---|
| | Group A (control) N=100 | Group B (T2DM) N=80 | Group C (DN) N=80 | | |
| *Age/Year:* | | | | | |
| X-¦SD | 51.62-¦8.93 | 52.18-¦7.97 | 53.68-¦7.40 | 1.453 | 0.236 |
| Range | 34.0-65.0 | 35.0 66.0 | 35.0-65.0 | | |
| | No. % | No. % | No. % | | |
| *Sex:* | 29 29.0 | 22 27.5 | 12 15.0 | $\xi^2$=5.417 | 0.067 |
| Male | 71 71.0 | 58 72.5 | 68 85.0 | | |
| Female | | | | | |
| | | | | 48.649 | <0.001 [1] |
| *HTN:* | 0.0 0.0 | 48 60.0 | 38 47.5 | 68.478 | <0.001 [2] |
| Positive | 100 100 | 32 40.0 | 42 52.5 | 2.514 | 0.113 [3] |
| Negative | | | | | |
| *Duration of HTN/Year:* | | | | | |
| X-¦SD | – | 3.31-¦1.51 | 9.24-¦4.99 | U=5.565 | <0.001 |
| Range | – | 1.0-6.0 | 1.0-20.0 | | |
| *MBP (mmHg):* | | | | | |
| | | | | 6.757 | <0.001 [1] |
| X-¦SD | 89.37-¦4.75 | 96.11-¦7.84 | 96.53-¦9.77 | 6.012 | <0.001 [2] |
| Range | 70.0-93.30 | 76.70-111.70 | 83.30-116.70 | 0.302 | 0.763 [3] |
| *Duration of DM/Year:* | | | | | |
| X-¦SD | – | 3.15-¦2.14 | 10.55-¦3.60 | U=10.212 | <0.001 |
| Range | – | 1.0-8.0 | 5.0-20.0 | | |
| *BMI kg/m $^2$:* | | | | 8.995 | <0.001 [1] |
| X-¦SD | 23.58-¦1.38 | 28.34-¦4.56 | 31.92-¦5.06 | 14.324 | <0.001 [2] |
| Range | 19.90-26.37 | 22.04-37.95 | 24.34-45.79 | 4.709 | <0.001 [3] |

X = Mean. Group A = Healthy control.
SD = Standard deviation. Group B = Type 2 diabetes without nephropathy (T2DM)
$X^2$ = Chi square test. Group C = Type 2 diabetes with nephropathy (DN)
U = Mann Whitney, 1= A#B. 2= A#C. 3= B#C.

Table (2): Comparison between cases and control regarding blood glucose and lipid profiles.

| | The studied groups | | | *t*-test | *p*-value |
|---|---|---|---|---|---|
| | Group A (control) N=100 | Group B (T2DM) N=80 | Group C (DN) N=80 | | |
| *FBS (mg/dL):* | | | | 13.234 | <0.001 [1] |
| X-¦SD | 88.13-¦8.11 | 139.68-¦32.60 | 155.88-¦36.52 | 15.751 | <0.001 [2] |
| Range | 69.0-100.0 | 93.0-210.0 | 120.0-320.0 | 2.960 | 0.004 [3] |
| *2HPP (mg/dL):* | | | | 12.176 | <0.001 [1] |
| X-¦SD | 121.48-¦10.82 | 176.28-¦39.07 | 203.07-¦44.35 | 16.076 | <0.001 [2] |
| Range | 95.0-137.0 | 120.0-264.0 | 150.0-350.0 | 4.055 | <0.001 [3] |
| *Hb A1 c%:* | | | | 26.425 | <0.001 [1] |
| X-¦SD | 3.37-¦0.63 | 7.67-¦1.34 | 9.63-¦2.11 | 28.217 | <0.001 [2] |
| Range | 2.40-4.60 | 6.0-12.0 | 5.60-13.0 | 7.020 | <0.001 [3] |
| *Cholesterol (mg/dL):* | | | | 9.600 | <0.001 [1] |
| X-¦SD | 185.68-¦15.08 | 230.20-¦39.22 | 257.92-¦46.92 | 13.237 | <0.001 [2] |
| Range | 145.0-205.0 | 140.0-320.0 | 199.0-360.0 | 4.055 | <0.001 [3] |
| *Triglyceride (mg/dL):* | | | | 10.750 | <0.001 [1] |
| X-¦SD | 96.18-¦5.32 | 135.88-¦32.68 | 139.73-¦40.22 | 9.616 | <0.001 [2] |
| Range | 82.0-105.0 | 100.0-210.0 | 100.0-300.0 | 0.664 | 0.507 [3] |
| *LDL (mg/dL):* | | | | 11.356 | <0.001 [1] |
| X-¦SD | 95.32-¦6.03 | 113.25-¦13.05 | 134.88-¦30.51 | 11.418 | <0.001 [2] |
| Range | 75.0-110.0 | 100.0-160.0 | 100.0-190.0 | 5.828 | <0.001 [3] |
| *HDL (mg/dL):* | | | | 7. 100 | <0.001 [1] |
| X-¦SD | 39.67-¦4.01 | 35. 10-¦4.62 | 36.0-¦7.63 | 3.807 | <0.001 [2] |
| Range | 30.0-45.0 | 20.0-45.0 | 25.0-75.0 | 1.203 | 0.231 [3] |

X = Mean, SD = Standard deviation. 1= A#B, 2= A#C, 3= B#C

Table (3): Comparison between cases and control regarding kidney functions and vitamin D level.

| | The studied groups | | | *t*-test | *p*-value |
|---|---|---|---|---|---|
| | Group A (control) N=100 | Group B (T2DM) N=80 | Group C (DN) N=80 | | |
| *Urea (mg/dL):* | | | | 1.484 | 0.139 [1] |
| X±SD | 26.89±7.0 | 28.53±7.75 | 30.78±6.67 | 3.779 | <0.001 [2] |
| Range | 12.0-40.0 | 15.0-40.0 | 15.0-40.0 | 1.968 | 0.051 [3] |
| *Creatinine (mg/dL):* | | | | 1.244 | 0.215 [1] |
| X±SD | 0.80±0.73 | 0.91±0.13 | 1.28±0.26 | 5.556 | <0.001 [2] |
| Range | 0.55-8.0 | 0.60-1.20 | 0.90-1.80 | 11.409 | <0.001 [3] |
| *EGFR (ml/min):* | | | | 0.634 | 0.527 [1] |
| X±SD | 95.39±11.60 | 93.92±17.85 | 64.46±14.83 | 15.270 | <0.001 [2] |
| Range | 80.20-119.0 | 62.50-13 8.29 | 29.51-95.89 | 11.345 | <0.001 [3] |
| *A/C ratio (mg/g):* | | | | U | |
| X±SD | 4.88±2.77 | 12.90±5.80 | 148.90±48.90 | 9.328 | <0.001 [1] |
| Range | 2.0-10.40 | 5.0-20.0 | 75.0-245.0 | 11.547 | <0.001 [2] |
| | | | | 10.981 | <0.001 [3] |
| *VITD (nmol/l):* | | | | U | |
| X±SD | 73.66±15.96 | 25.98±13.50 | 9.78±4.61 | 10.988 | <0.001 [1] |
| Range | 27.25-95.40 | 7.81-57.63 | 3.52-23.40 | 11.436 | <0.001 [2] |
| | | | | 8.612 | <0.001 [3] |

X = Mean. SD = Standard deviation.
U = Mann Whitney. A/C ratio = Albumin/creatinine ratio.
1 = A#B. 2= A#C. 3= B#C

There was a significant difference in the distribution of the Bb, bb and Bb+bb genotypes between the control and T2DM groups, which are associated with an increased risk of T2DM by 5.03-, 3.3- and 4.4-fold ($p$<0.001), respectively.

Additionally, there was a significant difference in the distribution of the Bb, bb and Bb+bb genotypes between the control and DN groups, which were associated with an increased risk of DN by 18.1-, 8.3- and 14.6-fold ($p$<0.001), respectively.

In addition, there was a significant difference in the distribution of the Bb, bb and Bb+bb genotypes between the T2DM and DN groups, and the diabetic patients with this genotype had a 3.60-, 2.52- and 3.32-fold increased risk of DN ($p$=0.001), respectively.

There was a significant difference in the distribution of the b allele between the control and T2DM groups, which was associated with a 3.3-fold increase in diabetes mellitus risk ($p$=0.001). There was also a significant difference between the control and DN groups, with 6.3 fold increase risk of diabetic nephropathy associated with the b allele ($p$<0.001). In addition, diabetic patients with this b allele had a 1.91 fold higher diabetic nephropathy risk ($p$=0.006).

*VDR BsmI genotypes and laboratory data of the T2DM and DN groups:*

The Bb, bb and Bb+bb genotypes were significantly associated with lower levels of vitamin D than the BB genotype ($p$<0.001), but there was no significant difference between these genotypes with respect to other laboratory parameters. In the DN group, the Bb, bb and Bb+bb genotypes were significantly associated with higher HbA 1 C, cholesterol, LDL ($p$<0.001), and TG ($p$<0.05) levels and lower levels of vitamin D ($p$<0.001) (Tables 5,6).

*Multivariate regression analysis:*

The MBP, HbA1C, A/C ratio, vitamin D level and BsmI genotype were independent risk factors for T2DM ($p$=0.042, $p$<0.001, $p$=0.049, $p$<0.001, $p$=0.004, respectively). Diabetes duration, BMI, HbA1C, BsmI genotype and vitamin D level were independent risk factors for DN ($p$=0.019, 0.023, 0.036, 0.035 and <0.001, respectively; Tables (7,8).

Table (4): BsmI genotypes and alleles among the studied groups.

| | Group A (control) N=100 | Group B (T2DM) N=80 | Group C (DN) N=80 | *t*-test | *p*-value | OR | 95% CI |
|---|---|---|---|---|---|---|---|
| | No. (%) | No. (%) | No. (%) | | | | |
| *BsmI gene:* | | | | | | | |
| BB# | 83 (83.0) | 42 (52.5) | 20 (25) | | | 1.00 | |
| Bb | 11 (11.0) | 28 (35) | 48 (60) | 19.881 $\chi^1$ | <0.001 | 5.03 | 2.3-11.1 |
| | | | | 62.284 $\chi^2$ | <0.001 | 18.1 | 8.0-41.0 |
| | | | | 13.251 $\chi^3$ | 0.001 | 3.60 | 1.8-7.3 |
| bb | 6 (6.0%) | 10 (12.5) | 12 (15) | 19.850 F1 | <0.001 | 3.3 | 1.1-9.7 |
| | | | | 65.239 F2 | <0.001 | 8.3 | 3.0-24.8 |
| | | | | 13.309 F3 | 0.001 | 2.52 | 0.9-6.8 |
| *BsmI gene:* | | | | | | | |
| BB# | 83 (83) | 42 (52.5) | 20 (25) | | | 1.00 | |
| Bb+bb | 17 (17) | 38 (47.5) | 60 (75) | 19.485 $\chi^1$ | <0.001 | 4.4 | 2.2-8.7 |
| | | | | 61.079 $\chi^2$ | <0.001 | 14.6 | 7.1-30.3 |
| | | | | 12.745 $\chi^3$ | <0.001 | 3.32 | 1.7-6.5 |
| *BsmI allele:* | N=200 | N=160 | N=160 | | | | |
| B# | 177 (90) | 112 (70) | 88 (55) | | | 1.00 | |
| b | 23 (10) | 48 (30) | 72 (45) | 19.215 $\chi^1$ | 0.001 | 3.3 | 1.9-5.7 |
| | | | | 51.354 $\chi^2$ | <0.001 | 6.3 | 3.7-10.7 |
| | | | | 7.680 $\chi^3$ | 0.006 | 1.91 | 1.21-3.0 |

$\chi$ = Chi squared test. F = Fisher's Exact test. # = Reference genotype
OR = Odds ratio. CI = Confidence interval. 1= A#B. 2= A#C. 3= B#C.

Table (5): Relationship between BsmI genotypes and laboratory data in group B (T2DM).

| | Genotypes in group B (T2DM) | | | | Test | *p*-value |
|---|---|---|---|---|---|---|
| | BB N=42 | Bb N=28 | bb N=10 | Bb+bb N=3 8 | | |
| *FBS (mg/dL):* | | | | | 1.205 k | 0.547 **1** |
| X±SD | 142.90±34.28 | 140.0±33.68 | 125.20±17.0 | 136.11±30.69 | 0.931 *t* | 0.355 **2** |
| *2HPP (mg/dL):* | | | | | 0.508 k | 0.776 **1** |
| X±SD | 179.19±40.46 | 177.64±42.29 | 160.20±15.64 | 173.05±37.75 | 0.699 *t* | 0.486 **2** |
| *Hb A1c%:* | | | | | 5.921 k | 0.052 **1** |
| X±SD | 7.26±0.87 | 8.10±1.78 | 8.20±1.09 | 8.13±1.62 | 2.954 *t* | 0.005 **2** |
| *Cholesterol (mg/dL):* | | | | | 0.921 k | 0.631 **1** |
| X±SD | 230.48±45.21 | 227.0±28.74 | 238.0±40.22 | 229.89±31.94 | 0.067 *t* | 0.947 **2** |
| *TG (mg/dL):* | | | | | 8.498 k | 0.014 **1** |
| X±SD | 138.57±34.43 | 140.36±32.57 | 112.0±7.89 | 132.89±30.81 | 0.774 *t* | 0.441 **2** |
| *LDL (mg/dL):* | | | | | 0.059 k | 0.971 **1** |
| X±SD | 113.10±12.15 | 113.57±15.21 | 113.0±11.35 | 113.42±14.15 | 0.111 *t* | 0.912 **2** |
| *HDL (mg/dL):* | | | | | 1.784 k | 0.410 **1** |
| X±SD | 34.95±5.36 | 34.79±3.55 | 36.60±3.92 | 35.26±3.69 | 0.299 *t* | 0.766 **2** |
| *Urea (mg/dL):* | | | | | 0.822 k | 0.663 **1** |
| X±SD | 28.14±7.82 | 29.36±7.26 | 27.80±9.37 | 28.95±7.76 | 0.461 *t* | 0.646 **2** |
| *Creatinine (mg/dL):* | | | | | 3.224 k | 0.199 **1** |
| X±SD | 0.92±0.11 | 0.87±0.16 | 0.94±0.11 | 0.89±0.15 | 1.023 *t* | 0.309 **2** |
| *EGFR (ml/min):* | | | | | 9.207 k | 0.010 **1** |
| X±SD | 92.15±13.76 | 101.17±21.64 | 81.09±12.95 | 95.88±21.51 | 0.914 *t* | 0.364 **2** |
| *A/C ratio (mg/g):* | | | | | 4.582 k | 0.101 **1** |
| X±SD | 14.05±5.76 | 11.07±5.16 | 13.20±6.91 | 11.63±5.65 | 1.810 U | 0.070 **2** |
| *VITD (nmol/l):* | | | | | 59.156 k | <0.001 **1** |
| X±SD | 36.40±10.31 | 14.47±3.31 | 14.47±4.0 | 14.47±3.45 | 7.691 U | <0.001 **2** |

K=Kruskal Wallis test. *t*=*t*-test.
U=Mann Whitney u test. F=Fisher's Exact test.
**1**=BB# Bb & bb genotypes **2**=BB# Bb+bb genotypes.

Table (6): Relationship between BsmI genotypes and laboratory data in group C (DN).

| | Genotypes in group C (DN) | | | | | |
|---|---|---|---|---|---|---|
| | BB N=20 | Bb N=48 | bb N=12 | Bb+bb N=60 | Test | *p*-value |
| *FBS (mg/dL):* | | | | | 7.252 k | 0.027 [1] |
| X±SD | 151.0±19.69 | 162.92±43.35 | 135.83±13.62 | 157.50±40.63 | 0.949 *t* | 0.346 [2] |
| *2HPP (mg/dL):* | | | | | 11.961 k | 0.003 [1] |
| X±SD | 197.90±21.83 | 212.54±52.57 | 173.83±11.11 | 204.80±49.68 | 0.856 *t* | 0.395 [2] |
| *Hb A1c%:* | | | | | 29.845 k | <0.001 [1] |
| X±SD | 7.42±1.59 | 10.19±1.69 | 11.07±1.67 | 10.37±1.71 | 6.795 *t* | <0.001 [2] |
| *Cholesterol (mg/dL):* | | | | | 27.856 k | <0.001 [1] |
| X±SD | 218.60±14.60 | 263.37±46.94 | 301.67±31.57 | 271.03±46.69 | 7.649 *t* | <0.001 [2] |
| *TG (mg/dL):* | | | | | 6.070 k | 0.048 [1] |
| X±SD | 122.40±23.23 | 147.04±47.14 | 139.33±20.24 | 145.50±43.09 | 2.283 *t* | 0.025 [2] |
| *LDL (mg/dL):* | | | | | 21.339 k | <0.001 [1] |
| X±SD | 112.0±24.19 | 145.0±29.75 | 132.50±22.11 | 142.50±28.66 | 4.654 *t* | <0.001 [2] |
| *HDL (mg/dL):* | | | | | 6.565 k | 0.038 [1] |
| X±SD | 37.80±3.27 | 35.96±9.46 | 35.17±3.43 | 35.80±8.58 | 1.015 *t* | 0.313 [2] |
| *Urea (mg/dL):* | | | | | 2.887 k | 0.236 [1] |
| X±SD | 28.50±7.32 | 31.04±6.79 | 33.50±3.40 | 31.53±6.32 | 1.787 *t* | 0.078 [2] |
| *Creatinine (mg/dL):* | | | | | 3.123 k | 0.210 [1] |
| X±SD | 1.27±0.30 | 1.31±0.25 | 1.17±0.24 | 1.28±0.25 | 0.185 *t* | 0.854 [2] |
| *EGFR (ml/min):* | | | | | 2.295 k | 0.317 [1] |
| X±SD | 66.19±19.76 | 63.05±12.99 | 67.42±12.67 | 63.93±12.94 | 0.478 *t* | 0.637 [2] |
| *A/C ratio (mg/g):* | | | | | 1.830 k | 0.401 [1] |
| X±SD | 156.40±35.12 | 145.75±57.61 | 149.0±26.70 | 146.40±52.71 | 1.194 U | 0.233 [2] |
| *VITD (nmol/l):* | | | | | 45.131 k | <0.001 [1] |
| X±SD | 16.63±3.18 | 8.19±2.44 | 5.87±1.90 | 7.73±2.51 | 6.311 U | <0.001 [2] |

K=Kruskal Wallis test. *t*=*t*-test.
U = Mann Whitney U-test. F=Fisher's Exact test.
1=Comparison between (BB, Bb & bb) genotypes groups. **2**=Comparison between (BB& Bb+ bb) genotypes groups.

Table (7): Multivariate regression analysis for independent risk factors of DM among diabetic group.

| | SE | Wald X2 | *p*-value | Odds ratio | 95% CI |
|---|---|---|---|---|---|
| BMI kg/m$^2$ | 0.54 | 2.19 | 0.139 | 2.23 | 0.77-6.47 |
| MBP (mmHg) | 0.13 | 4.13 | 0.042 | 1.31 | 1.01-1.70 |
| A/C ratio mg/g | 0.14 | 3.87 | 0.049 | 1.31 | 1.0-1.72 |
| Vit D (nmol/L) | 0.05 | 8.38 | <0.001 | 0.87 | 0.78-0.95 |
| BsmI gene | 0.39 | 8.40 | 0.004 | 3.13 | 1.45-6.77 |
| Hb A1c% | 0.93 | 23.48 | <0.001 | 6.13 | 2.14-13.56 |

Table (8): Multivariate regression analysis for independent risk factors for diabetic nephropathy among DN groups.

| | SE | Wald X2 | *p*-value | Odds ratio | 95% CI |
|---|---|---|---|---|---|
| DM duration (yrs) | 1.49 | 5.47 | 0.019 | 32.90 | 1.76-614.55 |
| BMI kg/m$^2$ | 0.20 | 5.18 | 0.023 | 1.56 | 1.06-2.30 |
| HbA1c % | 1.75 | 4.42 | 0.036 | 3.46 | 1.28-8.94 |
| LDL (mg/dL) | 0.04 | 0.77 | 0.380 | 0.97 | 0.89-1.04 |
| Vit D (nmol/L) | 0.047 | 33.27 | <0.001 | 0.76 | 0.69-0.84 |
| BsmI gene | 1.13 | 4.75 | 0.035 | 8.87 | 2.98-80.74 |

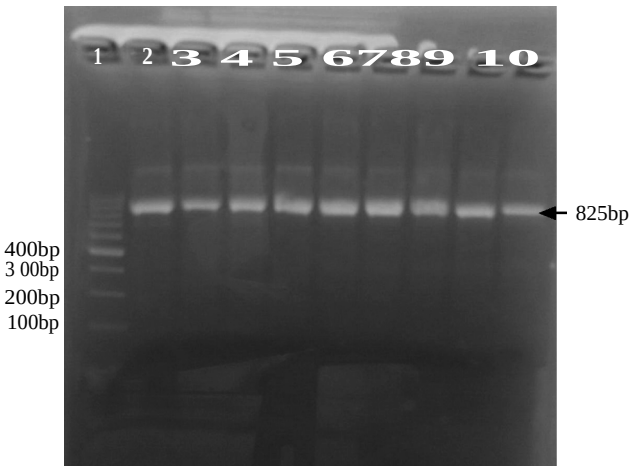


Fig. (1A): Agarose gel electrophoresis for BsmI genotypes before addition of restriction enzyme. Band size (825) correspond to BsmI gene.

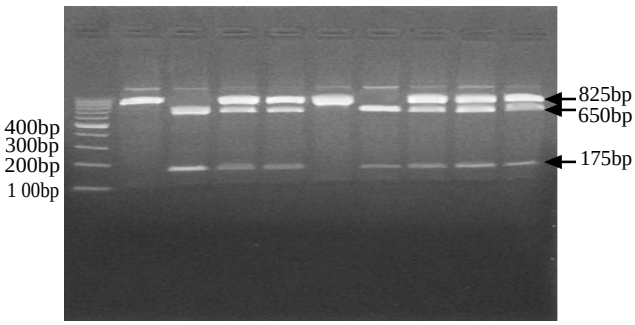


Fig. (1B): Agarose gel electrophoresis for BsmI genotypes after addition of restriction enzyme. Lanes: 1 (ladder) 2 and 6 (BB) band (825bp), lanes 3 and 7 (bb) band (650 and 175bp), lanes 4,5,8,9 and 10 (Bb) band (825,650 and 175bp).

## Discussion

The impact of vitamin D on insulin metabolism has been demonstrated [18]. Forouhi et al., [11] found that vitamin D deficiency affects kidney function. Previous studies reported the effects of vitamin D on glucose metabolism and indicated that the vitamin D level is controlled by various forms of VDRs in pancreatic ß-cells, skeletal muscle, and adipose tissue, which, in turn, may contribute to the genetic risk of type 2 diabetes [17,19]. Whether vitamin D deficiency is considered a risk factor in patients with DN remains unclear [11]. Thus, this study was conducted to study vitamin D status and single nucleotide polymorphisms (SNPs) in the VDR BSM1 gene and susceptibility to DN in Egyptian patients with type 2 diabetes.

Vitamin D deficiency plays a role in insulin resistance and the pathogenesis of type 2 diabetes mellitus [19]. Lower serum 25(OH)D concentrations are associated with higher risks for the development of type 2 diabetes mellitus [20].

In the present study, the mean values of vitamin D were significantly lower in diabetic and DN patients than in the control group and were lower in DN patients than T2DM patients. Accordingly, previous studies have shown that type 2 diabetic patients have more frequent vitamin D insufficiency and deficiency than control subjects [21,22]. The 25(OH)D values were lower in type 2 diabetes mellitus patients than in the control group, and poor vitamin D status (25(OH)D values) was associated with worse glycaemic control in type 2 diabetes mellitus patients [23,24]. 25(OH) D levels were lower in DN patients than in controls and were correlated with DN stage [25]. Vitamin D levels were low in Japanese patients with DN [26]. Experimental evidence showed that vitamin D may influence glycaemic control through modulation of the RAS signalling pathway and regulation of calcium ion traffic across ß-cells that directly affect insulin synthesis and secretion. In addition, vitamin D deficiency results in aberrant immune pathogenesis as an inflammatory reaction and subsequent insulin resistance [18].

Certain vitamin D binding proteins and genetic polymorphisms in the vitamin D receptor gene polymorphism may have effects on insulin secretion and glucose tolerance, which may lead to the genetic risk of type 2 diabetes and its complications [19].

The present study showed that the incidence of the BsmI gene was significantly different between the diabetic patients and controls. In our study, the Bb, bb and Bb+bb genotypes and the b allele significantly increased the risk of T2DM by 5.67-, 4.05-, 5. 13- and 3.86-fold, respectively. In agreement with these results, studies by Mukhopadhyaya et al., [27] and Zhang et al., [28] showed a significant association between BsmI gene polymorphisms and susceptibility to T2DM in an Indian and Chinese population, respectively. However, Zhang et al., showed that the BB+Bb genotype and B allele frequencies in the DM group were higher than those in the control group. In contrast to our study, other studies did not determine the role of BsmI at the rs1544410 polymorphism in T2DM [28, 10]. A study by Ogunkolade et al., [30] demonstrated that the VDR variant is a significant factor in VDR mRNA and VDR expression in PBMCs and confirmed an association between VDR polymorphisms and insulin secretion capacity. In addition, Mukhopadhyaya et al., [27] reported the association of the risk alleles for SNPs at rs1544410 with decreased insulin in T2DM, and these observations explain our results.

In this study, the Bb, bb and Bb+bb genotype and b allele frequencies were significantly greater in the DN group than in either the healthy control or T2DM group .In accordance with these results, Zhang et al., **[28]** showed an association between BsmI at the rs1544410 gene polymorphism and DN. However, in contrast to our results, he found that the BB+Bb genotype and allele B were more frequent in DN. Other studies showed no association between the BsmI gene polymorphism and DN in a Caucasian population **[31]** . These controversial results require further studies to elucidate the role of the VDR in microvascular complications, but the study by Tian et al., **[32]** revealed that the active form of vitamin D [1,25(OH)2D] has an inhibitory effect on the T helper (Th) 17 and Th1 responses. Thus, the variant vitamin D receptor may contribute to an inflammatory response in microvessels, which could explain our results. In addition, vitamin D hinders vascular smooth muscle cell growth and affects endothelial physiology differently in different ethnic populations. Therefore, it is suggested that VDR polymorphisms may influence the development of DN.

The present study investigated the association between different genotypes and clinical laboratory parameters, and there was an association between the Bb+bb genotype and higher MBP and HbA1C levels and between the Bb, bb and Bb+bb genotypes and lower vitamin D levels in T2DM patients. In the DN cases, there was an association between the Bb, bb and Bb+bb genotypes and higher HbA1C, cholesterol, TG and LDL levels, and there was a greater decrease in vitamin D levels compared with those in individuals with the BB phenotype.

In the present study, the multivariate regression analysis revealed that among the observed independent variables, the MBP, HbA1C, A/C ratio, vitamin D level and BsmI genotype are independent risk factors for T2DM. Diabetes duration, BMI, HbA1C, BsmI genotype and vitamin D level are independent risk factors for DN. Other studies showed that diabetes duration, systolic blood pressure (SBP) and HbA1c are associated with an increased risk of diabetic microvascular complications in type 2 diabetic patients **[33-35]** . Zhong et al., **[35]** also showed that patients with the B allele of the BSM1 gene exhibited a significantly lower risk of diabetic complications than patients without the B allele in a logistic regression analysis. In contrast, Capoluongo et al., **[36]** did not find an association between BsmI and diabetic complications in an Italian population.

To the best of our knowledge, this is the first study to investigate the association between diabetic complications and VDR polymorphisms in an Egyptian population. In conclusion, this study revealed that the vitamin D level and BsmI polymorphisms in the VDR gene can be used as susceptibility markers to predict the risk of DN in T2DM. However, the small number of subjects is a study limitation, and more studies are required to confirm these results with a larger sample size.

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# التنوع الجينى لمستقبلات فيتامين دى ونسبة فيتامين دى فى مرضى السكرى المصريين المصابين بقصور الكلى

\الخلفية: مرضى داء السكرى من النوع ٢ واعتلا ل الكلية السكرى لديهم متغيرات وراثية متعددة.

\الهدف: للتحقيق فى دور التنوع الجينى لمستقبلات فيتامين دى ومستوى مصل فيتامين دى فى المرضى المصريين الذين يعانون من اعتلال الكلية السكرى فى مرض السكرى من النوع ٢.

\المرضى وطرق \البحث: اشتمل هذا البحث ٤٠ مريضاً يعانون من داء السكرى من النوع الثانى، ٤٠ مريضاً يعانون من اعتلال الكلى السكرى و٨٠ من الاصحاء كمجموعة ضابطة. وتم عمل اختبارات الهيموجلوبين السكرى الميكروالبومين، تعدد الأشكال الجينى لمستقبلات فيتامين دى باستخدام تقنية تفاعل البوليمريز المتسلسل الزمنى، قياس مستوى مصل فيتامين دى باستخدام البكتروكيمولولسين فى كل المجموعات.

\النتائج: فى اشارة إلى النمط الجينى BB وجد ان النمط اليجنى BB,Bb ارتفا ع ذو دلالة احصائية فى اعتلال الكلى السكرى مقارنة بالمجموعة الضابطة ($p<0.001$) وتحمل مخاطر اعتلال الكلى السكرى بنسبة ٢٠.٤و١٧.٠ أضعاف على التوالى ومرضى السكرى ($p<0.01$) تحمل مخاطر اعتلال الكلى السكرى بنسبة ٣.٦ و٣.٣٢ أضعاف على التوالى. كما كان أليل b اعلى بكثير فى مجموعات اعتلال الكلى السكرى مقارنة بالمجموعة الضابطة ($p<0.001$). الحالات مع اعتلال الكلى السكرى لديها مستويات أقل من مصل فيتامين D قارنة بالمجموعة الضابطة وداء السكرى ($p<0.001$).

\الاستنتاجات: النمط الجينى BB,Bb واليل b فى التنوع الجينى لمستقبلات فيتامين دى هى عا مل خطر لنوع لمرض السكرى النوع ٢ واعتلال الكلى السكرى. أيضاً، هناك رابطة من نقص فيتامين D مع اعتلال الكلى السكرى فى مرض السكرى النوع ٢.