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Evaluation of Vitamin D Receptor *Taq I (rs731236)* and *Bsm I (rs1544410)* Gene Polymorphisms in Patients with Chronic Kidney Disease-mineral and Bone Disorder

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Abstract

BACKGROUND: Chronic kidney disease-mineral and bone disorder (CKD-MBD) is one of the main culprits of increasing morbidity and mortality in patients with stage: 3–5 CKD. Association between Vitamin D receptor (VDR) genetic polymorphisms and CKD-MBD has been inconsistent.

AIM: The aim of the study was to assess the association of VDR *Taq I (rs731236)* and *Bsm I (rs1544410)* gene polymorphisms with CKD; and with the development and progression of CKD-MBD.

METHODS: Sixty adult (43–56 years.) Egyptian CKD-MBD male patients (CKD stages: 3–5 with estimated glomerular filtration rate <60 mL/min 1.73 m^2) and 30 matched-pair healthy controls were recruited from Theodor-Bilharz Research Institute. *Bsm I* and *Taq I* polymorphisms of VDR gene were assessed using restriction fragment length polymorphism-polymerase chain reaction.

RESULTS: CKD-MBD patients having *Taq I* "tt" mutant gene had a significant decrease in serum 25 hydroxy Vitamin D and a significant elevation of plasma intact parathyroid hormone levels. Having the homotypic "tt" gene variant of VDR *Taq I* increased the susceptibility to CKD-MBD (Odds ratio [OR]: 19.6, CI: 4.3–89.9 $p < 0.01$) compared to having the wild "TT" or heterotype "Tt" genotype. Moreover, presence of VDR *Taq I* "tt" genotype increases OR of having 25 hydroxy Vitamin D deficiency in CKD-MBD patients 7.25 times (CI = 2.21–23.80; $p < 0.01$).

CONCLUSION: VDR *Taq I (rs731236)* "tt" genotype increases the susceptibility to CKD-MBD development and progression in Egyptian CKD patients. Moreover, the presence of *Taq I* "tt" genotype in CKD-MBD patients is independently associated with the risk of developing Vitamin D deficiency.

Introduction

Chronic kidney disease (CKD) is a global day epidemic. CKD with its complications form a public health and financial problem [1]. CKD-mineral and bone disorder (CKD-MBD) is one of the debilitating complications responsible for increased morbimortality associated with progressive CKD (stage: 3–5). In fact a high percentage of death in dialysis patients may be associated with poorly controlled CKD-MBD [2].

CKD-MBD includes one or a combination of the following: (i) Abnormalities in serum bone biomarkers-calcium, phosphorus, parathyroid hormone, or Vitamin D metabolites; and (ii) abnormalities in bone turnover or mineralization or (iii) vascular or soft-tissue calcification. Kidney-Disease Improving Global Outcome (KDIGO), 2017 recommended the monitoring of serum levels of 25-hydroxy-vitamin D [25 (OH) D], parathyroid hormone (PTH), calcium (Ca) and phosphorus (P) in diagnosis and management of CKD-MBD [3].

Despite the availability of global and regional guidelines to curtail the adverse clinical outcomes associated with CKD-MBD most chronic kidney disease (CKD) patients are still affected by the consequences of biochemical abnormalities of CKD-MBD, with an ever-increasing global prevalence and unacceptably high mortality rate [4]. Mounting evidence revealed that an interplay of genetic and environmental factors modulates the risk and progression of CKD and its accompanying mineral-bone disorders [5].

Alteration in Vitamin D metabolism is one of the key-players of CKD-MBD. The Vitamin D receptor (VDR) plays a key role in mediating the effects of biologically active Vitamin D (calcitriol). Calcitriol binds to VDR (D/VDR), orchestrating the maintenance of mineral homeostasis [6]. Moreover, VDR activation has a crucial renoprotective function in kidney disease through inhibition of renin-angiotensin system (RAS), decreasing proteinuria, and inhibiting apoptosis. It is plausible that variation in VDR will modulate calcitriol action. In CKD, impaired calcitriol/VDR (D/VDR)

interaction plays a critical role in development of CKD-MBD [7].

VDR gene, mapped on chromosome12q12-14, regulates VDR, hence, tightly coordinates renal calcium, phosphate reabsorption and parathyroid function, hindering the development of secondary hyperparathyroidism in CKD patients [6].

Since Vitamin D biosynthesis is regulated by VDR genes, their polymorphisms may alter bioavailability as well as target effects of Vitamin D metabolites. VDR Bsm I (rs1544410) and Taq I (rs731236) gene polymorphisms, present in intron 8 and exon 9, respectively, have been studied previously in association with CKD risk and progression; however, yielded controversial results [8], [9]. Moreover, conflicting results were found about the association between VDR gene polymorphisms and biochemical markers of CKD-MBD [10]. These two polymorphisms are considered to be good markers of disruption of the Vitamin D signaling pathway [11].

In an attempt to unravel the complexity behind the pathophysiologic mechanisms of CKD-MBD, several investigators studied the relationship between VDR polymorphisms and PTH/calcitriol axis with inconsistent findings [10]. The previous studies indicated that genotyping for VDR polymorphisms holds a great potential for the improvement of therapeutic approaches of CKD and management of CKD-MBD comorbidities.The screening for VDR variants helps to better address preventive strategies and improve management of CKD-MBD. Determination of candidate risk genes leading to CKD-MBD predisposition provide a better understanding of the pathogenesis of the disease and ensure optimal diagnosis and treatment [12].

The aim of the present work was to study the association of Vitamin D receptor gene polymorphisms *Bsm I and Taq I* with chronic kidney disease, in addition to assessment of its relationship with development and progression of chronic kidney disease-mineral bone disorder.

Subjects and Methods

1. Sixty consecutive CKD-MBD adult male patients were recruited from the Nephrology Department and out-patient clinic at Theodor-Bilharz Research Institute (TBRI) (Group 1). Using PASS 15 program for sample size calculation and according to previous literature [13], [14] the expected frequency of *Bsm I* B allele in CKD patients = 82% and healthy controls = 47.6%, and the expected frequency of *Taq I* T allele in CKD patients = 87% and in healthy controls = 55.3%. Thus, a sample size of 60 CKD patients and 30 healthy controls can detect the difference between two groups with power >80% and alpha error 0.05. The subjects were recruited from October 2017 till April 2018, male subjects were the preponderant gender in the study. Diagnosis of CKD-MBD was done according to presence of elevated serum P, Ca, iPTH or decrease in 25 (OH) D levels in stages 3–5 CKD patients (3). According to stages of CKD, based on estimated glomerular filtration rate (eGFR) (<15–<60 mL/min/1.73 m²), Group 1 was subdivided into two subgroups: 1a; Thirty CKD-MBD patients, CKD stages 3 and 4 (eGFR = 30–59 and 15–29 mL/min/1.73 m², respectively); and −1b; Thirty CKD-MBD patients with CKD stage 5 End-Stage Renal Disease (ESRD) (eGFR<15 mL/min/1.73 m²), on regular hemodialysis (3 times a week). Patients on parathormone therapy and patients with parathyroidectomy or chronic liver diseases were excluded from the study. Thirty (age-matched) male subjects were included as healthy controls (Group 2). Each patient and healthy examinee signed a written informed consent before the study. The study protocol and the consent from all participants were approved by the Ethical Committee and Institutional Review Board of TBRI, in accordance with the Declaration of Helsinki, 1975.
2. Demographic and clinical data were recorded. Routine laboratory investigations including serum creatinine (OSR6178, Beckman Coulter, USA), urea (OSR6134, Beckman Coulter, USA), uric acid (OSR6098, Beckman Coulter, USA), total calcium (OSR60117, Beckman Coulter, USA), phosphorus (OSR6122, Beckman Coulter, USA), alkaline phosphatase (OSR6204, Beckman Coulter, USA), and albumin (OSR6202, Beckman Coulter, USA) were assayed by Beckman AU480 (Beckman Coulter, USA). Ionized Ca was measured by ion-selective electrode. Serum 25 (OH) D (10699533, Siemens, USA) and plasma iPTH (10699154, Siemens, USA) were assayed by chemiluminescent immunoassay using *Siemens ADVIA Centaur auto analyzer Immunoassay System*. Calculation of eGFR (mL/min/1.73m^2) was done using the modification of diet in renal disease formula [15]: eGFR = 175 × (s. creatinine) $^{-1.154}$ × (age) $^{-0.203}$ × 0.742 (if female) × 1.212 (if black).
3. Assessment of VDR *BsmI (rs1544410) and TaqI (rs731236)* gene polymorphisms was performed by *PCR-RFLP* technique [14] as follows: Extraction of genomic DNA from EDTA blood using the QIAamp Blood Mini isolation kit (QIAGEN#51104), Germany, following manufacturer's instructions**.** Amplification

of the extracted DNA by polymerase chain reaction (PCR). The PCR program was as follows: initial denaturation at 94°C for 5 min., followed by 30 cycles of: denaturation at 94°C for 30 s, annealing at 60°C for 30 s, extension at 72°C for 1 min then final extension at 72°C for 5 min. The forward and reverse primers for the *BsmI* polymorphism (rs1544410): 5'CAAC CAAGACTACAAGTACCGCGTCAGTGA3'and 5'AACCAGCGGGAAGAGGTCAAGGG3', respectively, *TaqI* polymorphism (rs731236) was studied using forward 5'CAGAGCAT GGACAGGGAGCAA3' and reverse 5'GCAACTCCTCATGGCTGAGGTCTC3' primers. Digestion of the amplified product was performed by specific restriction endonuclease enzyme for *BsmI (rs1544410)* and *TaqI (rs731236)* within intron 8 and exon 9, respectively (Fermentas Fast Digest kits) (Thermo Fisher Scientific, Pittsburgh, PA, USA). Detection of VDR gene polymorphism *BsmI (rs 1544410)* and *TaqI (rs 731236)* was done by separation of PCR-amplification products using 3% agarose gel electrophoresis, (GeneRuler, Fermentas, Thermo Fisher Scientific), staining by ethidium bromide and ultraviolet transillumination (Figures 1 and 2).

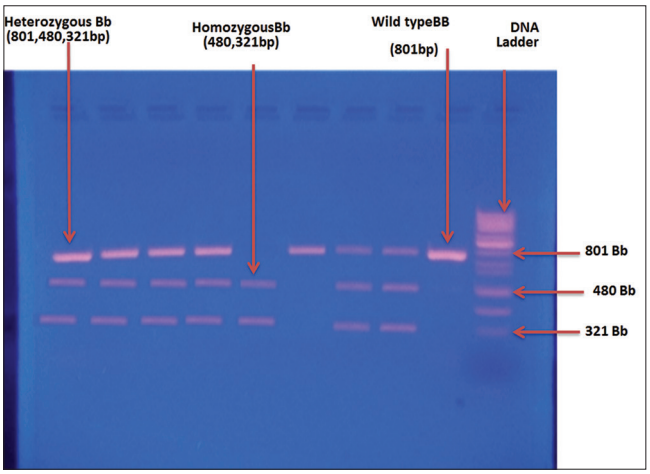

Figure 1: Detection of the bands of different genotypes of VDR BsmI gene polymorphism in agarose gel using ultraviolet transillumination. Wild or normal type (BB): one band (801 bp) heterozygous genotype (Bb): 3 bands (801, 480, and 321 bp) homozygous genotype (bb): 2 bands (480 and 321 bp)

In (Figure 1): PCR-RFLP of the VDR BsmI SNP (rs1544410) was performed: Digestion with the restriction enzyme BsmI, then gel electrophoresis in 3% agarose staining with ethidium bromide using a molecular weight marker of 50 bp. The resulting 801 bp amplified fragment contains a restriction site for BsmI restriction enzyme leading to:

- Presence of one band (801 bp), genotype was designated BB (wild or normal type)
- Presence of 3 bands (801, 480, and 321 bp), genotype was designated Bb (heterozygous genotype)

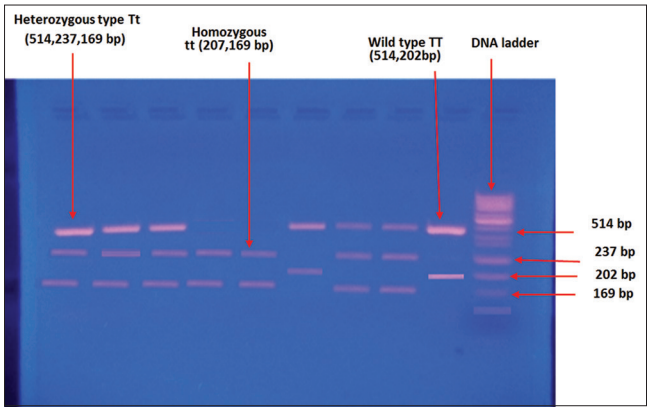

Figure 2: Detection of the bands of different genotypes of VDR TaqI gene polymorphism in agarose gel using ultraviolet transillumination. Wild or normal type (TT): two bands (514 and 202 bp) heterozygous genotype (Tt): three bands (514, 237 and 169 bp) homozygous genotype (tt): two bands (207 and 169 bp)

- Presence of 2 bands (480 and 321 bp) genotype was designated bb (homozygous genotype).

For VDR TaqI SNP (rs731236) as shown in (Figure 2) PCR-RFLP with the restriction enzyme TaqI was performed, then gel electrophoresis in 3% agarose stained with ethidium bromide using a molecular weight marker of 50 bp. The resulting 212 and 514 bp amplified fragments contain a restriction site for TaqI restriction enzyme leading to:

- Detection of the two bands (202 and 514 bp), genotype was designated TT (wild or normal type)
- Detection of the 3 bands (514, 237 and 169 bp), genotype was designated Tt (heterozygous genotype)
- Presence of the 2 bands (237 and 169 bp) genotype was designated tt (homozygous genotype).

Statistical methods

Statistical analysis was done using software version (V. 25.0, IBM Corp., USA, 2017–2018) of Statistical Package for the Social Sciences. Comparison of quantitative data (expressed as medians and inter-quartile range) was analyzed using Mann–Whitney U-test or Kruskal–Wallis test for differences between medians. Categorical variables of *VDR BsmI and TaqI* SNPs genotypes and phenotypes, presented as number and percentages were compared using Pearson's Chi-square (X^2) test; (Fisher exact test was used in observed frequency <5) and Z-test for comparison between two independent proportions. The clinical significance of *VDR BsmI and TaqI* gene SNPs in CKD-MBD patients and their association with Vitamin D deficiency was estimated by computing the odds ratio (OR) and 95% confidence intervals (CI) from logistic regression analysis. $p < 0.05$ was considered as a level of statistical significance for this study.

Table 1: Descriptive and comparative statistics of the various studied routine laboratory parameters in the different studied groups

Group parameter	Group 1 (patients' group) (n = 60)	Subgroup 1a (CKD-MBD 3 and 4) (n = 30)	Subgroup 1b (CKD-MBD 5) (n = 30)	Group 2 (control group) (n = 30)	H	p
Serum creatinine (mmol/L)	1.125 (0.65–2.25)	0.675 (0.55–0.9)	2.2 (1.625–2.675)	0.2 (0.1875–0.22)	−7.712	0.001
eGFR (mL/min/1.73 m²)	15 (7–27)	27 (19–35)	7.0 (6.0–9.0)	110 (98–117)	−7.708	0.001
Serum urea (mmol/L)	14.6 (9.6–25.3)	9.6 (8.6–11.4)	25.1 (16.8–30.8)	4.8 (3.8–5.6)	−7.705	0.001
Serum uric acid (μmol/L)	416 (356–475)	357 (297–416)	416 (357–535)	357 (238–416)	−3.233	0.001
Serum ALP (IU/L)	216 (128–487)	164 (104–324)	427 (179–545)	115 (96–150)	−4.272	0.001
Serum albumin (mmol/L)	0.57 (0.54–0.60)	0.55 (0.52–0.57)	0.58 (0.52–0.61)	0.61 (0.57–0.63)	−2.732	0.006
Serum calcium (mmol/L)	2.12 (1.99–2.29)	2.22 (2.12–2.47)	2.04 (1.89–2.22)	2.22 (2.04–2.37)	−1.384	0.166
Ionized calcium (mmol/L)	1.122 (1.07–1.27)	1.17 (1.07–1.22)	1.09 (0.99–1.29)	1.22 (1.09–1.29)	−1.464	0.143
Serum phosphorous (mmol/L)	1.64 (1.41–2.03)	1.61 (1.35–2.03)	1.64 (1.45–2.03)	1.25 (0.93–1.54)	−4.348	0.001
Plasma iPTH (ρmol/L)	34.7 (15.3–71.4)	15.5 (11.4–21.4)	71.1 (46.9–105.7)	6.1 (4.4–7.3)	−7.366	0.001
Serum 25 (OH) D (ηmol/L) (ng/ml)	44.9 (37.4–54.9) 18 (15–22)	44.9 (37.4–57.4) 18 (15–23)	44.9 (34.1–54.9) 18 (13.7–22)	49.9 (32.4–59.9) 20 (13–24)	−0.846	0.398

Data are expressed as median; 25th–75th percentiles; Group 1: Patients' group (patients with chronic kidney disease-mineral bone disorder stages 3, 4, 5 CKD), subgroup 1a; patients with chronic kidney disease-mineral bone disorder (stages 3,4 CKD), subgroup 1b; patients with chronic kidney disease-mineral bone disorder (stage 5 CKD), Group 2: Healthy control group, eGFR: Estimated glomerular filtration rate, ALP: Alkaline phosphatase, iPTH: Intact parathyroid hormone, Serum 25(OH) D: Serum 25-hydroxy Vitamin D, H: Kruskall–Wallis Test, p > 0.05: Non-significant difference, p ≤ 0.01: Highly significant difference, p ≤ 0.001: Highly significant difference.

Results

Results of the present study are summarized in Tables 1-4.

Descriptive and comparative statistics of the biochemical parameters (median; 25th–75th percentiles), of the studied groups (CKD-MBD [49; 45–53 y], subgroups1a [51; 45–56 y] and 1b [48; 44–52 y], and controls [47; 43–52 y]) are summarized in Table 1. There is significant impairment of renal functions evidenced by significant elevation of both serum urea and creatinine and a significant drop in eGFR in both CKD-MBD patients' subgroups (CKD stage 3–4: Subgroup 1a and CKD stage 5 [ESRD]: Subgroup 1b) as compared to control values ($p < 0.001$, respectively). There was a significant elevation of serum ALP, serum phosphorus (P), and plasma intact parathormone (iPTH) in both CKD-MBD subgroups (1a and 1b) as compared to control group values ($p < 0.001$, respectively), representing biochemical derangements due to bone and mineral disorders in chronic kidney disease patients. As regard serum 25 (OH) Vitamin D, a significant decrease in its level in subgroup 1a compared to the control value ($p < 0.01$); yet the decrease in its level was insignificant in subgroup 1b ($p > 0.5$).

Table 2 shows the distribution and comparison of VDR Bsm I and Taq I genotypic polymorphism and their allelic frequencies between group 1 (CKD-MBD patients) and Group 2 healthy controls and between subgroups 1a and 1b using the Chi-square test and Z test. As regards VDR Bsm I gene polymorphism, an insignificant difference in genotype and allelic frequencies was noticed between CKD-MBD patients' group (Group 1) and control subjects (Group 2) ($p > 0.05$, respectively).

However, genotype distribution and allele frequencies of TaqI polymorphism revealed a significant rise in the wild: "TT" genotype (23TT; 76.6%) and its allele: "T" (51T; 85%) frequencies in control as compared to CKD-MBD group (9TT; 15% and 34T; 28.3%; respectively) ($p < 0.001$, respectively). Meanwhile, in CKD-MBD patients, there was a significant rise in mutant "t" allele frequency (86t; 71.6%) in both the: homotype "tt" (35 tt; 58.3%) and heterotype "Tt" (16Tt; 26.7%) genotype distribution as compared to control group (9t; 15%, 2tt; 6.7% and 5Tt; 16.7%; respectively) ($p < 0.001$; <0.001 and <0.001; respectively) (Table 2). Homozygous mutant genotype (tt) is commoner with the progress of renal impairment in CKD-MBD subgroups with a significantly higher frequency in subgroup 1b (ESRD patients) compared to subgroup 1a (18tt; 60% vs. 17tt; 56.6%, respectively) ($p < 0.05$). Similarly,

Table 2: Distribution and comparison of *VDR BsmI and TaqI* genotypic polymorphism and their allelic frequencies between group 1 (CKD-MBD patients) and Group 2 (healthy control) and subgroup 1a and subgroup 1b using Pearson's' Chi-square test and Z test

Group VDR gene polymorphism	Group 1 CKD-MBD (n = 60)	Group 2 control group (n = 30)	Comparative tests		Subgroup 1aCKD -MBD 3&4 (n = 30)	Subgroup 1b CKD_ MBD 5 (n = 30)	Comparative tests	
VDR *BsmI* Genotype			Chi-square test				Chi-square test	
			X^2	p			X^2	p
BB (Wild type) % (n)	61.7 (37 BB)	66.6 (20 BB)	0.349	>0.05	63.3 (19 BB)	60.0 (18 BB)	0.753	>0.05
Bb (Heterotype) % (n)	28.3 (17 Bb)	26.7 (8 Bb)			30.0 (9 Bb)	26.7 (8 Bb)		
bb (Homotype) % (n)	10.0 (6 bb)	6.7 (2 bb)			6.7 (2 bb)	13.3 (4 bb)		
BsmI Alleles	(n = 120)	(n = 60)	Z test		(n = 60)	(n = 60)	Z test	
			Z	p			Z	p
B (Wild) % (n)	75.8 (91 B)	80 (48 B)	0.6283	>0.05	78.3 (47 B)	73.3 (44 B)	0.6397	>0.05
b (Mutant) % (n)	24.2 (29 b)	20 (12 b)	0.6283	>0.05	21.7 (13 b)	26.7 (16 b)	0.6397	>0.05
VDR TaqI Genotype			Chi-square test				Chi-square test	
			X^2	p			X^2	p
TT (Wild type) % (n)	15 (9 TT)	76.6 (23 TT)	35.234	<0.001	26.7 (8 TT)	3.3 (1 TT)	7.723	<0.05
Tt (Heterotype) % (n)	26.7 (16 Tt)	16.7 (5 Tt)			16.7 (5 Tt)	36.7 (11 Tt)		
tt (Homotype) % (n)	58.3 (35 tt)	6.7 (2 tt)			56.6 (17 tt)	60.0 (18 tt)		
TaqI Alleles	(n = 120)	(n = 60)	Z test		(n = 60)	(n = 60)	Z test	
			Z	p			Z	p
T (Wild) % (n)	28.4 (34 T)	85 (51 T)	7.1789	<0.001	35 (21 T)	21.7 (13 T)	1.6207	>0.05
t (Mutant) % (n)	71.6 (86 t)	15 (9 t)	7.1789	<0.001	65 (39 t)	78.3 (47 t)	1.6207	>0.05

Group 1: Patients' group (patients with chronic kidney disease-mineral bone disorder stages 3, 4, 5 CKD), subgroup 1a: patients with chronic kidney disease-mineral bone disorder (stages 3, 4 CKD), subgroup 1b: patients with chronic kidney disease-mineral bone disorder (stage 5 CKD), group 2: healthy control group, X2: Chi-square test, Z: Z test, p >0.05: Non-significant difference, p ≤ 0.05: Significant difference, p ≤ 0.001: Highly significant difference.

Table 3: Association of VDR *TaqI* gene polymorphism in group 1: CKD-MBD and group 2: Controls with OR and 95% confidence interval

VDR *TaqI* genotype	Group 1 (CKD-MBD patients) (n = 60)	Group 2 (healthy controls) (n = 30)	p	OR 95% CI (Min.-Max.)	Risk
TT (Wild type) % (n)	15 (9 TT)	76.7 (23 TT)	<0.001	0.05 (0.02–0.16)	Negative significant risk
Tt (Heterotype) % (n)	26.7 (16 Tt)	16.7 (5 Tt)		1.82 (0.6–5.6)	Non-risk
tt (Homotype) % (n)	58.3 (35 tt)	6.7 (2 tt)		19.60 (4.3–89.9)	Positive significant risk

VDR: Vitamin D receptor, Group 1: Patients with chronic kidney disease-mineral bone disorder, Group 2: Healthy control group, OR: Odds Ratio, 95% CI; 95% Confidence Interval, p<0.001; highly significant difference.

heterogenous Tt genotype was commoner in CKD-MBD subgroup1b with a significantly higher frequency as compared to subgroup 1a (11Tt; 36.7% vs. 5Tt; 16.7%, p < 0.05, respectively).

Table 3 shows the possible association between VDR TaqI gene polymorphism with CKD-MBD, evaluated using OR and 95% Confidence Interval. The OR calculation showed that the presence of the homotypic tt gene variant increased the risk of CKD-MBD by 19.6 folds (95% CI= 4.3–89.9) compared to the presence of the wild (TT) or the heterotypic (Tt) variants, respectively, (O.R 0.05, 95% CI = 0.02–0.16; and OR 1.82, 95% CI = 06–5.6, respectively).

Descriptive and comparative statistics of serum 25 (OH) D and plasma iPTH between various genotypes of VDR TaqI polymorphism in CKD-MBD patients' group and its subgroups using Kruskal–Wallis and Wilcoxon rank sum test revealed a significant decrease in 25 (OH)D levels in patients with homotype mutant (tt) compared to the patients having either heterotype (Tt) or wild type (TT) in Group 1, subgroup1a and subgroup 1b (p <0.001, <0.01, <0.01, respectively). A statistically significant difference was also observed between different genotypes regarding plasma iPTH being significantly higher in CKD-MBD patients with homotype "tt" TaqI genotype compared to wild "TT" and heterotype "Tt" in: Group 1, subgroups 1a and 1b (p < 0.01, respectively).

Descriptive and comparative statistics between different VDR BsmI and TaqI genotypes in different categories of serum 25-OH Vitamin D levels in Group 1 (CKD-MBD patients) and Group 2 (healthy controls) using the Chi-square test revealed that: Whereas a significant difference was observed regarding TaqI genotypes in different categories of serum 25 (OH) D levels in Group 1 (CKD-MBD patients'<0.05), the difference was insignificant in the control group.

Table 4 shows the association between the presence of different VDR TaqI genotypic polymorphisms and the deficiency of serum 25 (OH) D in CKD-MBD patients studied using the OR and the 95% confidence interval. Serum 25 (OH) D levels below <20 ng/mL (<49.92 nmoL/L) were considered as deficiency, while insufficiency levels ranged from 20 to 29 ng/mL (49.92–72.38 nmoL/L). The presence of TaqI homotypic "tt" gene variant increases the OR of having 25–OH Vitamin D deficiency (<20 ng/mL) and insufficiency (20–29 ng/mL) in CKD-MBD patients 7.25 times (95% CI = 2.21–23.80) and 3.86 times (95% CI = 1.1–12.81), respectively, compared to those patients having the wild TT and heterotypic Tt gene variant (Table 4). The presence of the mutant VDR BsmI types (homotype; bb, heterotype; Bb) was not associated with having 25 (OH) D deficiency or insufficiency in CKD-MBD patients.

Discussion

In this study, we evaluated the potential effect of two single nucleotide polymorphisms (SNP): *Taq I (rs731236) and BsmI (rs 1544410)* in the VDR gene on CKD risk and the development and progression of CKD-MBD in Egyptian CKD patients.

Results of the study revealed insignificant difference in genotype and allele distributions of *BsmI (rs154410)* VDR SNP between CKD-MBD patients and control group. Mutant heterotype "Bb" and homotype "bb" variants were not associated with vitamin D deficiency. In accordance with our findings, Nugroho *et al*. [16] revealed no association of *BsmI* "B" allele, "BB" and "bb" genotypes with CKD risk. The *BsmI* variants of VDR being silent SNP located at the intron region of the gene, do not change the amino acid sequence of encoded protein, and hence, they minimally affect susceptibility to CKD-MBD [8].

Meanwhile, in our studied CKD-MBD patients, significant rise in *TaqI (rs731236*) **"t"** allele, homozygous "tt**"** >and heterozygous **"**Tt**"** genotype frequencies was noticed, as compared to control group. Rise in *TaqI* **"**tt**"** frequency was significantly accentuated with progression of renal impairment (ESRD patients) as compared to subgroup 1a (CKD patients with stages: 3–4). Significant associations of VDR *TaqI* gene polymorphism with risk of diabetic nephropathy [17] and susceptibility to CKD [18] were reported in previous

Table 4: The association between the presence of different VDR *TaqI* genotypic polymorphism and deficiency of 25 (OH) Vitamin D in CKD-MBD patients (Group 1, n = 60) using odds ratio (OR) and 95% confidence interval (CI)

VDR TaqI genotype 25 (OH) D	Homotype (tt)	Heterotype (Tt)	Wild type (TT)	p	OR	95% CI (Min. -Max.)	Risk
Deficiency (<20 ng/mL; <49.92 nmol/L) % (n = 39)	74.5 (29)	15.5 (6)	10 (4)	<0.01	7.25	(2.21–23.80)	Positive significant risk
Insufficiency (21–29 ng/mL; 49.92–72.38 nmol/L) % (n = 20)	30 (6)	45 (9)	25 (5)		3.86	(1.1–12.81)	Positive significant risk

25 (OH):25-hydroxyvitamin-D; OR: Odds ratio, 95% CI; 95% confidence interval, p < 0.01: Significant difference.

studies, consistent with our results. Considering the pivotal role of VDR in renal protection, it is conceivable that *TaqI* VDR gene polymorphism leads to activation of RAS, resulting in angiotensin II-mediated renal damage, prompting inflammation, thus perpetuating progression of CKD to ESRD [7], [19], [20]. Contrary to our results, Zhou *et al*. [21] denied the presence of association between VDR *TaqI* "t" allele or "tt" genotype with CKD risk in Caucasian population in their meta-analysis study. It is noteworthy that, *TaqI* genotype distribution varies greatly across ethnic groups, hampering comparison of studies.

Moreover, in our study, the presence of *TaqI* homotypic "tt" gene variant increased the risk for developing CKD-MBD (OR; 19.6, CI: 4.3–89.9 p < 0.01), compared to having wild "TT**"**, or heterotypic "Tt**"** variants. In progressive CKD, impaired D/DR interaction, due to present *TaqI* VDR polymorphism, might be a key-player, among others, in development of CKD-MBD [5].

In addition, CKD-MBD patients with homozygous "tt" *TaqI* genotype described the highest iPTH level. In line with our results, Pourfarzam *et al*. [14] reported in their study that, the increased frequency of "tt" genotype for VDR *TaqI* was in CKD patients with the highest levels of iPTH. The Vitamin D receptor (VDR) gene (expressed in many tissues, namely, the parathyroid glands) is a crucial mediator of active vitamin D (calcitriol) biological activity. The main effect of calcitriol on PTH, through the VDR, is to decrease PTH gene transcription and D/DR inhibits parathyroid cell proliferation leading to a decrease in parathyroid hormone synthesis [22]. Therefore, it is plausible that mutation that inactivates the function of VDR (namely, *TaqI* genetic polymorphism in our study) could enhance proliferation of parathyroid cells and hence impairs the D/DR-mediated inhibition of PTH secretion, promoting hyperparathyroidism and leading to accentuation of progression of CKD-MBD [14]. This might interpret the association of a significant rise of PTH in CKD-MBD patients having VDR *TaqI* "tt" mutant genotypes as compared to those with wild "TT" or heterotype "Tt" genotypes, recorded in our study.

Furthermore, in our study, a significant drop in serum 25 (OH) D levels was recorded in CKD-MBD patients with *TaqI* "tt" mutant genotype. In addition, logistic regression analysis of our results revealed that *TaqI* "tt" genotype, in CKD-MBD patients, increased the risk of having 25 (OH) D deficiency compared to presence of wild **"**TT**"** or heterotype **"**Tt**"** gene variants. Previously, Li *et al*., [23] reported association of 25 (OH) D deficiency with risk of progression of CKD. Waziri *et al*. [10] in their study confirmed the association of VDR polymorphism with vitamin D deficiency in CKD patients. Patients with compromised VDR activity due to presence of *TaqI* "tt**"** genotype might be more susceptible to CKD-induced reduced renal 1-α hydroxylase, resulting in decreased active Vitamin D. It is plausible that SNP genetic polymorphism of VDR affects vitamin-D mediated signaling pathways, accentuating bone, and mineral disorders in CKD-MBD patients.

Hence, the presence of VDR TaqI homotype mutant "tt**"**, might be a candidate risk gene for development and progression of CKD-MBD in CKD patients, promoting hyperparathyroidism and Vitamin D deficiency.

Conclusion

This study revealed that VDR *BsmI (rs1544410)* gene polymorphism is not associated with susceptibility to CKD or development and progression of CKD-MBD in the studied Egyptian patients with CKD. However, having the gene variants of VDR *TaqI* (rs731236) tt homotypic genotype increased the susceptibility to CKD and CKD-MBD compared to having the wild **"**TT**"** or heterotype **"**Tt**"** genotypes. In addition, the presence of *TaqI* **"**tt**"** homotypic variant was found to be associated with secondary hyperparathyroidism in CKD-MBD patients. Moreover, risk of developing Vitamin D deficiency in CKD-MBD patients was associated with the presence of VDR *TaqI* "tt**"** genotype.

CKD-MBD is considered the nephrologist's endocrinopathy. Gaps of knowledge still exist, prompting the need for future longitudinal genetic studies to provide predictive, diagnostic, and prognostic information to assist in the management of CKD-MBD patients.

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