Association of BsmI Polymorphisms in the Vitamin D Receptor Gene Among Indonesian Population with Diabetic Kidney Disease

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ABSTRAK

Latar belakang: *penyakit ginjal diabetik (PGD) sebagai penyebab utama penyakit ginjal tahap akhir (PGTA), merupakan komplikasi dari diabetes mellitus (DM). Salah satu kondisi yang menjadi faktor risiko PGD yaitu defisiensi vitamin D, dimana polimorfisme reseptor vitamin D (VDR) disinyalir memiliki peran. Studi ini bertujuan untuk melihat adanya hubungan antara polimorfisme reseptor vitamin D (VDR) terhadap PGD, dan faktor yang memengaruhi hubungan tersebut.* ***Metode:*** *studi dilakukan secara potong lintang pada pasien DM Tipe 2 di poliklinik Penyakit Dalam RSUPN Dr. Cipto Mangunkusumo, Jakarta dengan rentang waktu November 2014 – Maret 2015. Subjek yang memenuhi kriteria penelitian dilakukan pengumpulan data berupa karakteristik subjek, pemeriksaan fisik, dan pemeriksaan darah (polimorfisme BsmI gen reseptor vitamin D). Pasien dengan penyakit akut dan berat dieksklusikan dari studi. Selanjutnya dilakukan analisis secara bivariat dan multivariat antar variabel.* ***Hasil:*** *dari 93 subjek penelitian, didapatkan 42 (45.2%) subjek tanpa PGD dan 51 (54.8%) subjek dengan PGD. Sebagian besar subjek memiliki genotip Bb yaitu sebesar 89.2%, serta tidak ada subjek yang memiliki genotip BB. Sebagian besar subjek memiliki alel b, yakni sebesar 55.4%. Tidak terdapat hubungan yang berbeda bermakna antara polimorfisme BsmI gen reseptor vitamin D dengan PGD (OR = 1.243; CI 95% 0.334-4.621; p = 0.751).* ***Kesimpulan:*** *Genotip Bb pada polimorfisme BsmI didapatkan sebesar 89.2% dan genotip bb sebesar 10.8%. Sebagian besar subjek memiliki alel b, yakni sebesar 55.4%. Tidak ditemukan adanya hubungan bermakna antara polimorfisme BsmI gen reseptor vitamin D dengan PGD. Durasi DM lebih dari lima tahun memengaruhi hubungan antara kedua variabel tersebut.*

Kata kunci: *polimorfisme, BsmI, gen reseptor vitamin D, penyakit ginjal diabetik, ras Indonesia-Malay.*

ABSTRACT

Background: *Diabetic kidney disease (DKD), as a common cause of end-stage renal disease (ESRD), is a chronic complication of diabetes mellitus (DM). It has been established that vitamin D deficiency is one of DKD risk factors, which may be related to vitamin D receptor (VDR) polymorphisms. This study aimed to analyze the association between VDR polymorphisms and DKD in Indonesian population, also risk factors that influence it.* ***Methods:*** *a cross-sectional study was conducted in Type 2 DM patients who visited internal medicine outpatient clinic at Dr. Cipto Mangunkusumo Hospital, Jakarta, from November 2014 until March 2015. Data collection*

includes characteristics of subjects and laboratory examination, including BsmI polymorphisms in the vitamin D receptor gene. Patients with acute and severe disease were excluded from the study. Bivariate and multivariate analyses were done. ***Results:*** *of 93 DM subjects, 42 (45.2%) subjects were without DKD and 51 (54.8%) subjects had DKD. Most of the subjects had the Bb genotype (89.2%), with no subject having the BB genotype. The proportions of the B and b alleles were 44.6% and 55.4%, respectively. There is no association between BsmI polymorphisms in the vitamin D receptor gene and DKD (OR = 1.243; CI 95% 0.334-4.621; p value = 0.751).* ***Conclusion:*** *the profile of BsmI polymorphisms in the vitamin D receptor gene in the Indonesian population were genotypes Bb (89.2%) and bb (10.8%). There was no association between BsmI polymorphisms in the vitamin D receptor gene and DKD. Duration of DM more than five years influenced the association between those variables.*

Keywords: *polymorphisms, BsmI, vitamin D receptor (VDR) gene, diabetic kidney disease, Indonesian–Malay race.*

INTRODUCTION

Diabetic kidney disease (DKD) is a common cause of end-stage renal disease (ESRD). Data from the Indonesian Renal Registry (2011) revealed that the 25% etiology of patients who have undergone hemodialysis is DKD.[1] Factors that are associated with DKD include blood glucose control, hypertension, dyslipidemia, duration of diabetes mellitus (DM), high body mass index, age, sex, ethnicity, vitamin D deficiency, high-sodium diet, high-protein diet, and smoking.[2–7]

Vitamin D has an anti-calcium effect by inhibiting the renin-transcription process, angiotensin II, the renal-inflammation process, and albumin excretion. It also prevents podocyte damage, glomerulosclerosis, and transformation from kidney epithelial cells into mesenchymal cells.[8] Current studies on the association between vitamin D deficiency and DKD are still controversial. Those controversial results may be related to genetic factors, which are vitamin D receptor (VDR) polymorphisms. The VDR polymorphism is affected by the *Cdx2, ApaI, BsmI, FokI,* and *TaqI* genes, whereas the Asian population is mostly affected by *ApaI, FokI*, and *Cdx2*.[9]

Several studies have reported the association between *BsmI* polymorphisms in the vitamin D receptor gene and DKD in various populations. Zhang et al. (2012) study revealed an association between *BsmI* polymorphisms in the vitamin D receptor gene and DKD in Han Chinese population, while Vedralova et al. (2012) study in Caucasians showed the opposite result.[10,11] However, the association between *BsmI* polymorphism in the vitamin D receptor gene and DKD has not been investigated in the Indonesian population. Based on the current evidence, we aimed to analyze the association between *Bsml* polymorphism in the vitamin D receptor gene and DKD particularly in Indonesian population. In terms of secondary objective, we aimed to investigate other risk factors that influence the association between main variables. By knowing the potential risk factor of *BsmI* polymorphism in the vitamin D receptor gene, it is hoped that the number of cases and morbidity of DKD can be prevented.

METHODS

A cross-sectional study was conducted to understand the association of *BsmI* polymorphisms in the vitamin D receptor gene with DKD among the Indonesian–Malay race. The subjects of this study were patients who came to the internal medicine outpatient clinic, Dr. Cipto Mangunkusumo Hospital, Jakarta, from November 2014 until March 2015. The inclusion criteria were patients with type-2 DM, of Indonesian–Malay population, who signed the informed consent form. Patients with urinary tract infection or fever, who were pregnant or in their menstrual period, had undergone hemodialysis or peritoneal dialysis, had used nonsteroid anti-inflammatory drugs (NSAIDs), and postexercise were excluded. This study has been approved by the Ethical Committee

of Faculty of Medicine Universitas Indonesia (Reference no. 756/UN2.F1/ETIK/2014).

Data collection included subjects' characteristics, physical examination, and laboratory examination, including *BsmI* polymorphisms in the vitamin D receptor gene. 5 mL of venous blood was collected in a non-fasting state and added to an EDTA-anticoagulated container. BsmI polymorphisms were measured using polymerase chain reaction with high resolution melt analysis. Since the diabetic kidney disease was defined from albuminuria, the mid-stream random urine was collected in a urine collector. Albuminuria was measured using *Nyocacard U-albumin* with sandwich immunometric assay technique.

The data were then analyzed by SPSS v.16. Participants' characteristics were reported in percentages for categorical data, mean (standard deviation), or median (range) for continuous data. A chi square test was used to analyze the association between *BsmI* polymorphisms in the vitamin D receptor gene and DKD. Also, logistic regression analysis was performed to investigate the role of other risk factors that influence the association between *BsmI* polymorphisms in the vitamin D receptor gene and DKD. Bivariate and multivariate analyses were presented with confidence interval 95% and considered statistically significant if the p value was <0.05.

RESULTS

93 subjects were recruited for the present study. There were 51 subjects (54.8%) with DKD and 42 subjects (45.2%) without DKD. The characteristics of the subjects, which include demography and laboratory examination results, are presented in **Table 1**.

Table 1. Characteristics of the subjects.

	DKD (n = 51)	Without DKD (n = 42)
Sex, n (%)		
- Male	28 (66.7)	14 (33.3)
- Female	23 (45.1)	28 (54.9)
Age, median (range)	61 (46-73)	61.5 (45–85)
Body mass index (kg/m^2), mean (SD)	26.08 (3.93)	25.40 (3.84)
Duration of DM, n (%)		
- > 5 years	39 (63.9)	22 (36.1)
- ≤ 5 years	12 (37.5)	20 (62.5)
Systolic BP (mmHg), median (range)	137 (88–180)	130 (98–173)
Diastolic BP (mmHg), median (range)	74 (52–100)	75 (51–94)
eGFR (mL/min/1.73 m^2), mean (SD)	43.46 (22.46)	73.51 (23.23)
Urea (mg/dL), median (range)	39 (17–145)	25.5 (13–54)
Creatinine (mg/dL), median (range)	1.5 (0.7–9.6)	0.9 (0.6–1.9)
Fasting blood glucose mg/dL), median (range)	133 (79–365)	121.5 (75–285)
2-hours postprandial blood glucose (mg/dL), median (range)	199 (60–521)	180.5 (80–479)
HbA1c (%), median (range)	7.5 (5.4–11.1)	7.2 (5.9–11.4)
Total cholesterol (mg/dL), mean (SD)	187.35 (44.66)	175.48 (35.34)
Triglyceride (mg/dL), median (range)	115.5 (30–447)	95.5 (52–272)
HDL cholesterol (mg/dL), mean (SD)	50.57 (18.77)	53.57 (12.76)
LDL cholesterol (mg/dL), mean (SD)	117.88 (40.46)	108.00 (29.47)

DKD: Diabetic kidney disease; DM: Diabetes mellitus; BP: Blood pressure.

The proportion of Genotype and Allele of *BsmI* Polymorphisms in Vitamin D Receptor Gene are presented in **Table 2**. The majority of the subjects had the Bb genotype (89.2%), and no subject had the BB genotype. The percentage of the b allele was 55.4%.

Table 2. Genotype and allele of the subjects.

	Frequency n (%)
Genotype	
- Bb	83 (89.2)
- bb	10 (10.8)
Allele	
- B	83 (44.6)
- b	103 (55.4)

Association of *BsmI* Polymorphisms in Vitamin D Receptor Gene with DKD

There is no association between *BsmI* polymorphisms in the vitamin D receptor gene and DKD (OR = 1.234; CI 95% 0.334–4.621; p = 0.75). There is also no significant association between allele B or allele b on *BsmI* polymorphisms in the vitamin D receptor gene and DKD (OR = 1.043; CI 95% 0.584–1.866; p = 0.89). (**Table 3** and **Table 4).**

Risk Factors of DKD

Several factors have significant associations with DKD, such as duration of DM being more than five years (p value = 0.015), blood

Table 3. Association of BsmI polymorphisms in vitamin D receptor gene with DKD.

	DKD n (%)	Without DKD n (%)	Total n (%)	OR	95% Confidence Interval	
					Min	Max
Bb	46 (55.4)	37 (44.6)	83 (100)	1.243	0.334	4.621
bb	5 (50)	5 (50)	10 (100)			

Table 4. Association between B allele of BsmI polymorphisms in vitamin D receptor gene and DKD.

	DKD n (%)	Without DKD n (%)	Total n (%)	OR	95% Confidence Interval	
					Min	Max
B	46 (55.4)	37 (44.6)	83 (100)	1.043	0.584	1.866
b	56 (54.4)	47 (45.6)	103 (100)			

Table 5. Risk factors of DKD.

	DKD n (%)	Without DKD n (%)	OR	95% Confidence Interval	
				Min	Max
Genotype					
- Bb	46 (55.4)	37 (44.6)	1.243	0.334	4.621
- bb	5 (50)	5 (50)			
Duration of DM					
- > 5 years	39 (63.9)	22 (36.1)	2.955	1.218	7.167
- ≤ 5 years	12 (37.5)	20 (62.5)			
Body mass index					
- Overweight and Obese	40 (59.7)	27 (40.3)	2.020	0.806	5.062
- Normal	11 (42.3)	5 (57.7)			
Blood pressure					
- Hypertension	48 (62.3)	29 (37.7)	7.172	1.883	27.319
- Without hypertension	3 (18.8)	13 (81.2)			
Blood glucose control					
- Uncontrolled	40 (55.6)	32 (44.4)	1.023	0.378	2.769
- Controlled	11 (55)	9 (45)			
Dyslipidemia					
- Dyslipidemia	28 (57.1)	21 (42.9)	1.217	0.537	2.760
- Without Dyslipidemia	23 (52.3)	21 (47.7)			
Blood pressure control					
- Uncontrolled	23 (62.2)	14 (37.8)	1.643	0.705	3.829
- Controlled	28 (50)	28 (50)			
Kidney function					
- eGFR < 60	41 (75.9)	13 (24.1)	9.146	3.531	23.690
- eGFR ≥ 60	10 (25.6)	29 (74.4)			

pressure (p value = 0.001), and kidney function (p value < 0.001). From logistic regression analysis, confounding factors had effects on the association between *BsmI* polymorphisms in the vitamin D receptor gene and DKD with crude OR of 1.243 (95% CI 0.334–4.621) and adjusted OR of 1.410 (95% CI 0.335–2.296). Duration of DM being more than five years influenced the association of *BsmI* polymorphisms in the vitamin D receptor gene and DKD. The results are presented in **Table 5** and **Table 6**.

Table 6. Crude OR and Adjusted OR Genotype as Risk Factor of DKD and Effect from Other Risk Factors

	OR	95% CI	
Genotype Bb	Crude OR: 1.243	0.334–4.621	
Adjusted			
(+) eGFR	1.137	0.248–5.216	8.5%
(+) Hypertension	1.058	0.219–5.121	6.9%
(+) Duration of DM	1.449	0.278–7.551	37%
(+) Body Mass Index	1.525	0.283–8.217	5.2%
(+) BP Control	1.410	0.260–7.652	7.5%

DISCUSSION

The studied participants were type-2 DM patients who came to the Internal Medicine Outpatient Clinic, Dr. Cipto Mangunkusumo Hospital, Jakarta. Most of the subjects were female with ages ranging from 45 to 85 years old. Dewi et al.[12] study and Indra et al.[13] study showed similar participants; most of their subjects were female. The duration of DM of most subjects was more than five years (65.6%), and most subjects had comorbidities, such as hypertension, elevated body mass index, and dyslipidemia. These findings resembled Indra et al.[13] study, which had 82.8% patients with hypertension and most of them with more than five years' duration of DM. Insulin resistance in type-2 DM patients causes an increase in angiotensin II, inflammatory mediator release, which causes endothelial damage, and hyperglycemia, which increases sodium reabsorption in renal tubules. Hence, plasma volume will increase, which leads to hypertension.[14]

Even though hypertension was the major comorbidity among type-2 DM patients in this study, the median blood pressure in both groups was less than 140/90 mmHg, since 81.7% of subjects had already taken antihypertensive drugs such as ACE inhibitors or ARBs. More than half of the participants belonged to the DKD group (54.8%). This finding was related to the higher number of patients with comorbidities such as hypertension, dyslipidemia, overweight, poor glycemic control (HbA1c median = 7.45), and more than five years of DM. A total of 82.8% subjects had a higher body mass index, with a mean of 26.08 from the DKD group and 25.40 from the non-DKD group. It was found that obesity leads to oxidative stress conditions, which cause endothelial damage and decrease adiponectin levels. Such conditions result in renal podocyte damage.[15]

Most subjects with DKD had an estimated glomerular filtration rate (eGFR) of less than 60 mL/min/1.73 m^2 (75.9%). Albuminuria in DKD causes reduced renal function by triggering chemokine expression and activating complements of renal tubules, which cause infiltration of inflammatory cells in interstitial and fibrogenesis. In the end, reduced renal function may have resulted in an ESRD state.[16]

Based on the results of this study, 10.8% of the subjects had the bb genotype, 89.2% had the Bb genotype, and none had the BB genotype. There were 44.6% subjects with the B allele and 55.4% with the b allele. This result was different from Zhang et al.[10] study, which showed the majority of the bb genotype among the participants. On the other hand, Vedralova et al.[11] showed the BB genotype for most of the patients. Based on these previous studies, it can be concluded that genotype differences are influenced by race. Indonesian native citizens, most of whom come from the Malay race, are different from Han Chinese, who belong to the Mongoloid race, and are also different from the Caucasian race.

Data suggested an elevated number of DKD among BB genotypes (adjusted OR 1.410), but the confidence interval was above 1 (CI 95% 0.260–7.652). This result contradicted Zhang et al. (2012),[10] who revealed a significant correlation between *BsmI* polymorphisms in the vitamin D receptor gene and DKD among Han

Chinese population.

Besides *BsmI*, vitamin D receptor polymorphism was influenced by other investigated genes, such as *TaqI, ApaI*, and *FokI.* Zhang et al.[10] study conducted among Han Chinese population stated that there is no association between *ApaI* polymorphisms in the vitamin D receptor gene and DKD in the same population. A study from Arababadi et al. (2010) found that there was no significant association between *ApaI* and *TaqI* polymorphisms and DKD. Arababadi et al. (2010) found that vitamin D receptor gene polymorphisms have an association with DM, but not with DKD.[17] Vedralova et al.[11] also found that the vitamin D receptor-gene polymorphism that has an association with DKD is *FokI*.

In the case of the separation of alleles B and b, no significant association was found between the B allele of *BsmI* polymorphisms in the vitamin D receptor gene and DKD (p value = 0.89). This result was different from Zhang et al.[10] study, which found that allele B has a significant association with DKD among Han Chinese population. According to logistic regression analysis, more than five years' duration of DM has the greatest influence on the association of *BsmI* polymorphisms in the vitamin D receptor gene and DKD. Another study also reported that a longer duration of DM increases the prevalence of DKD and increases the proteinuria.[18]

To the best of our knowledge, this is the first study to analyze the proportion and association of *BsmI* polymorphisms in vitamin D receptors with DKD in the Indonesian–Malay race. This study provides a preliminary understanding of vitamin D receptor-gene polymorphisms and the influence of genetic factors in the therapeutic response of DKD. However, this study lacks the measurement of both vitamin D and vitamin D receptor levels of the subjects which might affect the association between variables. Hence, there were several confounding factors affecting the association between the *BsmI* gene-receptor vitamin D and DKD. Also, this study analyzed only the association of the *BsmI* gene as one of the vitamin D receptor-gene polymorphisms. Therefore, this study was not able to describe the entirety of vitamin D receptor-gene polymorphisms. This still warrants further studies.

CONCLUSION

The profiles of *BsmI* polymorphisms in the vitamin D receptor gene in the Indonesian–Malay race were genotypes Bb (89.2%) and bb (10.8%). There was no association between *BsmI* polymorphisms in the vitamin D receptor gene and DKD, which might be due to the influence of genetic factors among different populations. In addition, the duration of DM being more than five years influenced the association between those variables.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

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