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Original Article

Association between the CYP24A1 rs2762939 polymorphism and vascular calcification in Indonesian patients with chronic kidney disease on hemodialysis

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Abstract

Vitamin D plays a key role in mineral metabolism, and its dysregulation contributes to vascular calcification, a major complication of chronic kidney disease–mineral and bone disorder (CKD–MBD) in patients undergoing hemodialysis with CKD. The *CYP24A1* gene encodes 24-hydroxylase, the enzyme responsible for degrading active vitamin D metabolites and its polymorphisms, particularly rs2762939, have been linked to variability in vitamin D status and coronary artery calcification. The aim of this study was to assess the association between the rs2762939 polymorphism of *CYP24A1* and vascular calcification in Indonesian patients with CKD undergoing maintenance hemodialysis. A case–control study was conducted in 92 hemodialysis patients, including 46 with vascular calcification and 46 without. Genotyping of the rs2762939 polymorphism was carried out using PCR–RFLP, and the amplified products were separated by electrophoresis on 4% agarose gel. The frequency of vascular calcification was found to be significantly higher in patients with diabetes mellitus than in the control group (19 (82.6%) vs 4 (17.4%)), whereas in non-diabetic patients the frequency of vascular calcification was lower compared with controls (27 (39.1%) vs 42 (60.9%)). A statistically significant association between CKD etiology and vascular calcification was observed (p=0.001). The prevalence of vascular calcification was lower among carriers of the mutant C allele (45%) compared with the G allele (51.4%), although this difference was not statistically significant (OR=0.77; 95%CI: 0.38–1.56; p=0.592). The rs2762939 polymorphism of the *CYP24A1* gene was not significantly associated with vascular calcification in Indonesian patients with CKD undergoing maintenance hemodialysis. Further studies with larger, ethnically diverse cohorts and integration of vitamin D status are needed to clarify the genetic contribution of CYP24A1 and related pathways to vascular calcification.

Keywords: Chronic kidney disease, CYP24A1, rs2762939 polymorphism, vascular calcification, Indonesia

Introduction

Chronic kidney disease (CKD) is a progressive and irreversible condition characterized by structural or functional kidney damage lasting more than three months. Diagnosis is based on a reduced glomerular filtration rate (GFR) and the severity of albuminuria [1,2]. Chronic kidney disease – mineral and bone disorder (CKD-MBD) is a complex syndrome, frequently observed in patients with impaired renal function, that results from a disruption in mineral homeostasis due to declining kidney function [3]. CKD-MBD is one of the common complications in CKD patients undergoing hemodialysis and among the characteristics, vascular calcification is the hallmark of

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CKD-MBD [4]. One of the factors that also affects the process of vascular calcification is the amount or level of vitamin D [4]. The concentration of 1,25(OH)2D is tightly regulated by 1-hydroxylase and the catabolic enzyme 24-hydroxylase gene (*CYP24A1)*. *CYP24A1* expression is induced by 1,25(OH)2D and 25 (OH)D and is one of the most inducible genes in humans [5]. The high inducibility of *CYP24A1* is most likely an important factor in the large therapeutic window of vitamin D [5].

CYP24A1 is a cytochrome P450 group gene, family 24, subfamily A, polypeptide 1 with a cytogenetic location of 20q13.2 [6]. The *CYP24A1* encodes the enzyme 24-hydroxylase, which catalyzes the degradation of the active form of vitamin D, 1,25(OH)2D. Loss of *CYP24A1* function leads to increased serum concentrations of 1,25(OH)2D associated with hypercalcemia, hypercalciuria, and low plasma parathyroid hormone (PTH) levels [7]. Several genetic studies have reported that single nucleotide polymorphisms (SNPs) in vitamin D pathway genes including the *CYP24A1* are associated with impaired serum vitamin D levels (8,9,10,11). Among polymorphisms in *CYP24A1* that have been assessed in different populations, only SNP rs2762939 remained associated with vascular calcification [5]. A previous study demonstrated that C allele of rs2762939 may contribute to elevated levels of both 1,25 (OH)2D and total vitamin D [12]. This C allele is also associated with a significantly reduced risk of coronary artery calcification [12]. Another previous study examining the association between *CYP24A1* polymorphisms and coronary artery calcification across three independent populations confirmed a significant correlation between the C allele of rs2762939 and reduced calcification, further supporting the protective role of vitamin D in coronary artery calcification [5].

Given the limited data on whether the rs2762939 polymorphism of *CYP24A1* is associated with vascular calcification in CKD patients undergoing regular hemodialysis, and the absence of such data in Indonesia; therefore, the aim of this study was to investigate the association between the rs2762939 polymorphism of *CYP24A1* and vascular calcification in Indonesian patients with CKD on regular hemodialysis.

Methods

Study design and setting

A case–control study was conducted to examine the association between *CYP24A1* gene polymorphisms and vascular calcification in Indonesian patients with CKD undergoing maintenance hemodialysis. The investigation was conducted between June and September 2024 at Rasyida Kidney Hospital, Medan, North Sumatera, Indonesia.

Patients and criteria

This study included patients with CKD aged ≥18 years who had been undergoing maintenance hemodialysis for more than three months and provided written informed consent. The duration of hemodialysis was obtained from medical records, along with demographic and clinical variables including age, etiology of CKD, and laboratory parameters such as serum calcium and phosphate levels. Patients with incomplete or missing data were excluded from the analysis. Vascular calcification was assessed by measuring carotid intima–media thickness (CIMT) using real-time B-mode ultrasonography. CIMT was defined as the distance from the intima–lumen interface to the media–adventitia boundary, and vascular calcification was considered present when CIMT exceeded 1.0 mm. A total of 46 patients were included in each study group: the case group comprised CKD patients with vascular calcification, whereas the control group comprised CKD patients without vascular calcification.

Sample size and sampling method

The sample size was determined using the Lemeshow formula, assuming a Type I error (α) of 0.05 and a Type II error (β) of 0.20, corresponding to a statistical power of 80%. The calculation, based on the formula for comparing two proportions, indicated a minimum requirement of 29 participants per group. To enhance statistical robustness and compensate for potential dropouts or incomplete data, the final sample size was increased to 46 participants per group, yielding a total of 92 participants. A purposive sampling strategy was applied, with subjects recruited according to predefined inclusion criteria until the target sample size was achieved.

Clinical and laboratory data

Clinical and demographic data were obtained from patients' medical records. Variables collected included age, sex, duration of hemodialysis, and the underlying etiology of CKD. Information regarding comorbidities such as diabetes mellitus, hypertension, and cardiovascular disease was also recorded. Laboratory parameters were retrieved from routine blood tests performed at the hospital's clinical laboratory including calcium and phosphate levels.

Blood samples

The DNA of the blood samples from the subjects had been isolated and stored in Integrated Laboratory Universitas Sumatera Utara as stored biological materials owned by Riri Andri Muzasti as part of previous project [13]. Only DNA samples with concentrations ranging from 0.5 to 1.8 ng/µL (measured using a NanoDrop spectrophotometer) were included in the analysis.

CYP24A1 gene polymorphism assessment

Polymorphism rs2762939 of the *CYP24A1* gene was analyzed using polymerase chain reaction-restriction fragment length polymorphism (PCR–RFLP). The primers used were forward 5′-CCAAACGTGCTCATCATCTG-3′ and reverse 5′-ATCAAACACATCCAGTGGAAA-3′. PCR amplification was carried out with an initial denaturation at 95 °C for 5 minutes, followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 54 °C for 30 seconds, and extension at 72 °C for 1 minute. A final extension step was performed at 72 °C for 10 minutes. The PCR products were digested with the restriction enzyme Sau96I-Cfr13I (Thermo Scientific, Waltham, MA, USA), which selectively recognizes and cleaves the G allele. Genotypes were resolved by electrophoresis: homozygous wild type (GG) was identified by two fragments (45 and 79 bp), homozygous mutant (CC) by a single 124 bp fragment, and heterozygous (GC) by three fragments at 45, 79, and 124 bp [12].

Statistical analysis

Data were processed and analyzed using IBM SPSS Statistics (IBM Corp., Armonk, NY, USA). Univariate analysis was performed to summarize the distribution of baseline characteristics. The association between genotypes and alleles of the *CYP24A1* rs2762939 polymorphism and vascular calcification in CKD patients undergoing maintenance hemodialysis was assessed using the Chi-square (χ^2) test. The strength of association was expressed as odds ratios (ORs) with 95% confidence intervals (95%CI). Deviation from Hardy–Weinberg equilibrium in both groups was evaluated using HardyWeinbergTesting (HW_TEST) software. A p-value <0.05 was considered statistically significant.

Results

Characteristics of patients

A total of 92 CKD patients were included in this study, 46 patients with vascular calcification (case group) and 46 patients without vascular calcification (control group). There was no significant difference between age, duration of hemodialysis, calcium and phosphorus levels between the case and control groups. The characteristics of patients from both groups are presented in (**Table 1**). The frequency of diabetes was higher in case than control groups (41.3vs 8.7%) resulting there was a statistically significant association of the etiology of CKD (diabetes or non-diabetes) with vascular calcification with a p=0.001 (**Table 1**). The other characteristics were not statistically significantly associated with vascular calcification between groups (**Table 1**).

Genotype and allele distributions of *CYP24A1* polymorphism

The distributions of genotypes and alleles of the rs2762939 polymorphism of the *CYP24A1* are presented in (**Table 2**). The GG was the most common genotype in both groups, of which 30 samples (54.5%) in the case group and 25 (45.5%) in the control group. Based on Hardy-Weinberg equilibrium calculation, there was no significant deviation of genotype frequency both in case and control groups (p>0.05). The frequency of G allele was greater in the case group (74;51.4%) than the control group (70;48.6%). In contrast, the frequency of C allele was greater in the control group (22;55%) than in the case group (18; 45%) (**Table 2**).

Table 1. Comparison of characteristics between chronic kidney disease (CKD) patients with and without vascular calcification

Category	Case (n=46) n (%)	Control (n=46) n (%)	*p*-value
Age (years)			
Young (<60)	23 (50)	18 (39.1)	0.401
Elderly (≥60)	23 (50)	28 (60.9)	
Duration of hemodialysis (months)			
≤100	1 (2.2)	2 (4.3)	0.390
101–149	36 (78.3)	30 (65.2)	
150–199	7 (15.2)	13 (28.3)	
≥200	2 (4.3)	1 (2.2)	
Etiology of CKD			
Diabetes	19 (41.3)	4 (8.7)	0.001
Non-diabetes	27 (58.7)	42 (91.3)	
Calcium level (mg/dL)			
Hypocalcemia-normal (≤10.5)	40 (87)	42 (91.3)	0.738
Hypercalcemia (>10.5)	6 (13)	4 (8.7)	
Phosphorus level (mg/dL)			
Hypophosphatemia-normal (≤4.5)	8 (17.4)	5 (10.9)	0.549
Hyperphosphatemia (>4.5)	38 (82.6)	41 (89.1)	

Associations between genotypes and alleles of *CYP24A1* with vascular calcification

There was no significant association between the *CYP24A1* rs2762939 genotype and vascular calcification in hemodialysis patients. The odds ratios for the CC and GC genotypes were 0.35 (95%CI: 0.029–4.246) and 0.60 (95%CI: 0.05–7.01), respectively (**Table 2**). Similarly, there was no significant association between the C*YP24A1* rs2762939 allele and vascular calcification. Although the mutant C allele had a lower likelihood of vascular calcification compared to the G allele (OR=0.77; 95%CI: 0.38–1.56), this association was not statistically significant (p=0.592) (**Table 2**). Overall, the genotype and allele distributions did not differ significantly between patients with and without vascular calcification.

Table 2. Genotype and allele frequencies of rs2762939 polymorphism of *CYP24A1* gene

Genotype or allele	Case (vascular calcification) n (%)	Control (no vascular calcification) n (%)	OR (95%CI)	*p*-value	*p*-value HWE case	*p*-value HWE control
GG	30 (54.5)	25 (45.5)			0.822	0.186
GC	14 (41.1)	20 (58.9)	0.60 (0.05–7.01)	0.684		
CC	2 (66.7)	1 (33.3)	0.35 (0.02–4.24)	0.410		
G	74 (51.4)	70 (48.6)	0.77 (0.38–1.56)	0.592		
C	18 (45.0)	22 (55.0)				

Discussion

This study represents the first pilot investigation in Indonesia evaluating the association between *CYP24A1* gene polymorphism, particularly rs2762939, and vascular calcification. Consistent with previous findings, the homozygous wild-type GG genotype was the most prevalent in both the case and control groups. Prior studies have reported that the rs2762939 polymorphism of the *CYP24A1* gene is associated with reduced coronary artery calcification (3,8). In line with these observations, our results suggested that carriers of the C allele might have a potential protective effect against vascular calcification; however, the association did not reach statistical significance (OR 0.77; 95%CI: 0.38–1.56; p=0.592).

A study tested the association of *CYP24A1* polymorphism and coronary artery calcification CAC in three populations, the Amish Family Calcification Study (AFCS), the Genetic Epidemiology Network of Arteriopathy (GENOA), and the Penn Coronary Artery Calcification (PennCAC) cohorts found that rs2762939 C allele consistently showed association with reduced coronary artery calcification, with meta-analysis yielding a highly significant result ($p=2.9\times10^{-6}$) [5].

Another study reported an association between the *CYP24A1* rs2762939 polymorphism and acute coronary syndrome (ACS), showing that the GG genotype was significantly associated with an increased risk of ACS, independent of vitamin D levels [15]. Shen and colleagues further demonstrated that the *CYP24A1* gene encodes the primary enzyme responsible for the catabolism of 1,25(OH)2D and 25 (OH)D, and that genetic heterogeneity in vitamin D homeostasis may contribute to the pathophysiology of coronary artery calcification [5]. Consistent with these findings, previous research indicated that although the rs2762939 polymorphism was not directly associated with the prevalence or extent of coronary artery disease (CAD), carriers of the C allele exhibited significantly lower rates of coronary calcification, whereas GG homozygotes had substantially greater calcification [12]. In the context of vascular calcification, coronary artery calcium levels have been shown to be influenced by this common *CYP24A1* variant, with several SNPs linked to arterial calcium deposition and related cardiovascular diseases [16].

Carotid artery calcification is a well-recognized predictor of cardiovascular morbidity and mortality in patients with CKD. The underlying mechanisms of vascular calcification are complex and multifactorial. Chronic inflammation, reflected by elevated interleukin-6 (IL-6) levels, has been correlated with an increased risk of carotid artery calcification in CKD patients undergoing maintenance hemodialysis. In addition, genetic factors have been implicated, with studies reporting an association between *KLOTHO* gene polymorphisms and carotid artery calcification in this patient population [17,18].

This study has several limitations. First, the ethnic composition of the participants was not assessed. Considering the high genetic diversity of the Indonesian population, allele frequencies may vary among different ethnic groups and could influence the observed association with vascular calcification. Second, the relatively small sample size may have limited the statistical power to detect modest associations and reduced the generalizability of the findings. Third, vitamin D levels, which are biologically relevant and potentially correlated with vascular calcification, were not measured. Future studies should therefore recruit larger, ethnically stratified cohorts, incorporate measurements of vitamin D status, and extend the analysis to additional *CYP24A1* polymorphisms as well as other genes involved in vitamin D metabolism to further elucidate genetic determinants of vascular calcification.

Conclusion

In CKD patients undergoing maintenance hemodialysis, the *CYP24A1* rs2762939 polymorphism was not significantly associated with vascular calcification. Although a lower prevalence of vascular calcification was observed among carriers of the C allele compared with the G allele, the difference did not reach statistical significance. Further studies with larger and more diverse cohorts are needed to clarify the genetic contribution of CYP24A1 to vascular calcification.

Ethics approval

This study was approved by the Health Research Ethics Committee of Universitas Sumatera Utara (Approval No. 258/KEPK/USU/2024).

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None to declare.

Competing interests

All the authors declare that there are no conflicts of interest.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

Declaration of artificial intelligence use

The authors confirm that no artificial intelligence (AI) tools or methodologies were employed at any stage of this study, including data collection, analysis, visualization, or manuscript preparation. All work presented in this study was conducted manually by the authors without the assistance of AI-based tools or systems.

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