ORIGINAL ARTICLE

Is there an association between alpha 2-Heremans-Schmid glycoprotein (AHSG) gene Thr256Ser polymorphisms with aortic calcification in regular hemodialysis patients in Medan, Indonesia

Riri Andri Muzasti[1], Suhardjono[2], Bambang Purwanto[3], Rosita Juwita Sembiring[4]

[1]Division of Nephrology and Hypertension, Department of Internal Medicine, Universitas Sumatera Utara, Medan, [2]Division of Nephrology and Hypertension, Department of Internal Medicine, University Indonesia, Jakarta, [3]Division of Nephrology and Hypertension, Department of Internal Medicine, University Negeri Surakarta, Solo, [4]Department of Clinical Pathology, Universitas Sumatera Utara, Medan; Indonesia

ABSTRACT

Aim Alpha2-Heremans-Schmid glycoprotein (AHSG), a circulating plasma protein, plays an essential role in bone and vascular mineralization. The impact of AHSG gene polymorphisms on aortic calcification in haemodialysis patients was inconsistent. We performed this study to clarify precise association among AHSG gene Thr256Ser single-nucleotide polymorphisms and aortic calcification.

Methods Patients on stable regular haemodialysis treatment for more than thirty months were included in a cross-sectional study at Rasyida Renal Hospital Medan. Lateral spine X-rays were performed to evaluate the aortic calcification. Genotyping for the polymorphisms was carried out using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) techniques.

Results Aortic calcification was detected in 69.8% of patients. From 106 patients, 49 patients (46.2 %) had CC (Thr/Thr), 54 (51.0%) had CG (Thr/Ser) and three (2.8%) patients had GG (Ser/Ser) polymorphism. The proportion of patients with heterozygous or homozygous G allele (CG and GG genotypes) is more likely (91.2%) to have aortic calcification. The bivariate analysis showed that Thr256Ser polymorphism (G allele) was associated with increased risk for aortic calcification (PR=2.03; 95% CI 1.48–2.80; p<0.001). However, overall results from multivariate analysis showed that Fetuin-A level <204 pg/mL (PR=22.0; 95% CI 3.32–145.91; p=0.001) and IL-6 level ≥53.05 mg/dL (PR=19.50; 95% CI 2.87–132.41; p=0.002) were the major risk factors for the occurrence of aortic calcification.

Conclusion AHSG Thr256Ser gene polymorphism showed an association with aortic calcification in regular haemodialysis patients, but Fetuin-A and IL-6 have a dominant role in the development of aortic calcification.

Key words: alpha-2-HS-glycoprotein, vascular calcification, haemodialysis

Corresponding author:
Riri Andri Muzasti
Division of Nephrology and Hypertension.
Department of Internal Medicine, Faculty of Medicine, Universitas Sumatera Utara
Dr. Mansyur 5 Medan, Indonesia
Phone: +62 81 260 556 872;
Fax: +62 61 821 6264;
E-mail: riri.andri@usu.ac.id
ORCID ID: https://orcid.org/0000 0001 7834 0740

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INTRODUCTION

The morbidity and mortality in Chronic Kidney Disease (CKD) is mainly caused by cardiovascular disease. This cardiovascular event is characterized by vascular calcification, which leads to ischemia of myocardium, insufficiency of cardiac valvular arrhythmias, and even stroke. In the early stages of CKD and young haemodialysis (HD) patients, the incidence of vascular calcification is high (1). The calcification occurs due to abnormal deposition of calcium and phosphate in the vascular tissue. Vascular calcification can occur both in tunica intimal and medial. The medial calcification, Mockenberg's Sclerosis, is typical in CKD patients and contributes to the mortality of CKD patients (1,2).

In CKD, there are some inhibitors of the calcification process, Alpha 2-Heremans-Schmid glycoprotein (AHSG) is one of them (1). AHSG is the circulating inhibitor of hydroxyapatite formation, which can lead to ectopic calcification, especially in vascular calcification. Its circulating levels area decline in CKD patients significantly, and so that has a contribution to the calcification process (3,4). The way AHSG works in inhibiting ectopic calcification is by binds to a growth factor and inactivates transforming growth factor-β (TGF-β) and bone morphogenic protein, which is the major part of mineralized bone, and also prevents the precipitation of basic calcium phosphate. AHSG also inhibits the apoptosis of vascular smooth muscle cells (VSMCs) at the cellular level (2). Single nucleotide polymorphisms (SNPs) of the AHSG gene (Thr256Ser) might affect serum AHSG levels and associated with cardiovascular events in CKD patients (5,6). Unfortunately, The role of AHSGgene polymorphisms to aortic calcification in HD patients was inconsistent. Stenvinkel et al. in Sweden found Thr256Ser polymorphism; namely, the Ser/Ser genotype (G allele) in the AHSG gene reduced the levels of Fetuin-A in HD patients (7). This decline increases the risk of cardiovascular disease mortality due to vascular calcification and non-cardiovascular mortality due to inflammation or other diseases. In contrast to the researcher above, Zeidan et al. (2012) in Egypt found no association between Thr256Ser AHSG gene polymorphism with decreased levels of Fetuin-A HD patients (5).

Aim of this study was to analyze whether the AHSG gene Thr256Ser polymorphisms have a relationship with vascular calcification in regular haemodialysis patients in Medan, Indonesia.

PATIENTS AND METHODS

Patients and study design

This study was a population-based, cross-sectional analytic study with 106 samples of regular haemodialysis patients at the Outpatient Polyclinic of Rasyida Renal Hospital Medan. Sample collecting was done from February to December 2018. The inclusion criteria were CKD patients who underwent haemodialysis for at least thirty months, and the patients were willing to take part in the research with laboratory and lateral lumbar X-ray examination. All patients signed an informed consent. The exclusion criteria were incomplete medical records.

The patients were then interviewed to determine the history of diseases, such as type 2 diabetes mellitus and the duration of haemodialysis. An anthropometry examination was performed to assess the body mass index (BMI), as well as laboratory tests such as serum phosphate level, serum calcium, fetuin A level, interleukin-6 level (IL-6), and albumin level.

The investigation was approved by the Health Research Ethical Committee of Medical Faculty of Universitas Sumatera Utara/H. Adam Malik General Hospital.

Methods

The Interleukin-6 level was measured using the turbidimetry method (Architect Multiagent CRP Harmonization 6K26-30, Indonesia). Calcium test was performed by measuring serum level based on the cresolphthalein complexone method (ADVIA 03932883 RevA, Indonesia), Phosphate test was performed by measuring level based on the phosphomolybdate method (ADVIA 024220623 Reva, Indonesia), while albumin level test was performed by measuring serum level based on the bromcresol green (Architect 7D5322, Indonesia).

AHSG analysis was performed by measuring serum level based on the ELISA technique (Qayee Bio Vendor Human Fetuin-A ELISA, Shanghai).

The DNA extraction was examined by polymerase chain reaction (PCR) technique (Esco Swift TM Maxi Thermal Cycler, Singapore). DNA extracted from blood leukocytes from the whole blood taken from the purity and concentration of genomic DNA extract was determined using a spectrophotometer 1000 nanodrop Thermo Scientific (Ther

mo Fisher Scientific, USA). The PCR-RFLP technique was used to detect the Thr256Ser gene.

Plain abdominal lateral radiography examination was performed to see the presence of aortic calcification. Calcification of large blood vessels, such as the aorta assessed using the lateral abdominal or lateral lumbar radiography (X-Ray Acoma Tube Toshiba, Japan) (8). The difference between intimal and medial calcifications was determined by radiographic examination, irregular and patchy distribution on imaging was classified as intimal calcification, appearance rail-road track like was classified as medial calcification (9).

An Abdominal Aortic Calcification (AAC) score is a scoring system to assess calcification (10). The assessment uses a segment of the abdominal aorta which is in front of the one to fourth lumbar vertebrae, where grade 0 means no calcification deposition in front of the vertebrae; grade 1 calcification deposition less than 1/3 of the aortic wall; grade 2 1/3-2/3 from the aortic wall of calcification; grade 3 more than 2/3 calcified aortic walls. By using this grading system, the score starts from a minimum of 0 to a maximum of 24 points. The patient has no calcification if the score is 0, mild calcification scores if the score is 1-4, and severe calcification if the score is above 4 (10).

Statistical analysis

The characteristic of regular haemodialysis patients' data was presented as a percentage for categorical data. Numerical data are displayed as mean ± standard deviation (SD) if the data is normally distributed and displayed as median (min-max), if the data distribution is not normal. The normality test was performed using Kolmogorov Smirnov. The $\chi 2$ or Fisher's Exact Test was used for the comparison of proportions between the two groups of variables. The logistic regression test was used to understand the association between risk factors and aortic calcification. Correlations between variables were assessed by Spearman correlation or Pearson correlation coefficient. The p-value was considered significant if it was less than 0.05.

RESULTS

The majority of patients were male, 65 (61.3%), with the mean age 53.9±11.4.

The median (min-max) time of the dialysis was 57.5 (34-237) months. The majority of patients had hypertension as comorbid disease 76 (71.7%) and had underweight-normal BMI 75 (70.8%).

The median (min-max) calcium level was 9.8 (8.0-10.9) mg/dL, mean phosphate level of 5.4±0.6 mg/dL, median (min-max) fetuin A level, of 235.0 (78–756) pg/mL were found. From the examination of Thr256Ser polymorphisms, the majority of patients had CG genotype 54 (50.95), followed by CC genotype, 49 (46.2%), and GG genotype was found in three (2.8%) patients. The median (min-max) AAC score was 7 (0-22). A large number of patients had calcification,74 (69.8%) (Table 1).

Table 1. The characteristic of regular haemodialysis (HD) patients

Variables	No (%) of patients	Mean ± SD	Median (min-max)
Gender		-	-
Male	65 (61.3)		
Female	41 (38.7)		
Age (year)		53.9 ± 11.4	57 (21-78)*
<60	68 (64.2)		
≥60	38 (35.8)		
Duration of HD (months)		69.4 ± 34.6	57.5 (34-237)*
<60	57 (53.8)		
≥60	49 (46.2)		
Comorbid disease		-	-
Diabetes Mellitus	23 (21.7)		
Hypertension	76 (71.7)		
Heart disease	17 (16.0)		
Stroke	11 (10.4)		
BMI (kg/m²)		24.2 ± 4.1	23.8 (16.7-42.7)*
Underweight-normal	75 (70.8)		
Overweight-obese	31 (29.2)		
Calcium (mg/dL)		9.7 ± 0.7	9.8 (8.0-10.9)*
≤ 9.5	43 (40.6)		
>9.5	63 (59.4)		
Phosphate (mg/dL)		5.4 +0.6	5.5 (4.0 – 6.8)
≤ 5.5	57 (53.8)		
>5.5	49 (46.2)		
Fetuin-A (pg/ml)		260.0 ± 119.4	235.0 (78 – 756)
IL-6 (pg/ml)		99.6 ± 115.5	70.7 (25.4 – 898.0)
Albumin (g/dL)		3.9 ± 0.3	3.9 (2.9 – 4.9)*
<3.9	62 (58.5)		
≥ 3.9	44 (41.5)		
Polymorphisms, n (%)			
CC Genotype (Thr/Thr)	49 (46.2)		
CG Genotype (Thr/Ser)	54 (50.9)		
GG Genotype (Ser/Ser)	3 (2.8)		
AAC Score		6.6± 5.9	7 (0 -22)*
Vascular calcification			
No Calcification	32 (30.2)		
Calcification	74 (69.8)		
Calcification degree			
No	32 (30.2)		
Mild	14 (13.2)		
Severe	60 (56.6)		
Calcification location			
No	32 (30.2)		
Tunica intima	5 (4.7)		
Tunica media	24 (22.6)		
Tunica intima & media	45 (42.5)		

*Data distribution is not normal

Figure 1 shows the results of restriction enzyme analysis (SacI) on the AHSG gene. C Allele did not contain SacI enzyme so that it is not cut off as 405 bp fragment, while G allele containing SacI enzyme will be cut off as 193 and 212 bp fragment. There were three AHSG gene genotypes, Thr/Thr (C homozygous allele/genotype C), Thr/Ser (G heterozygous allele/CG genotype), and Ser/Ser (G homozygous allele/GG genotype).

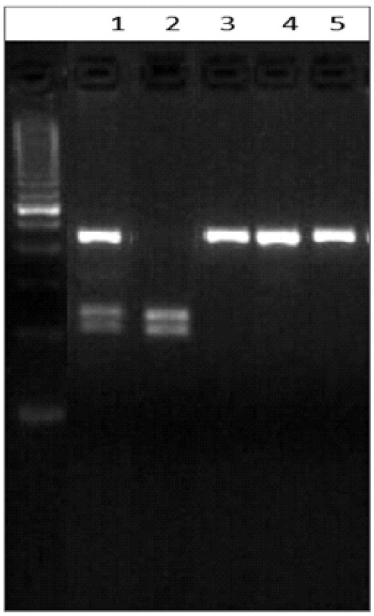

Figure 1. The RFLP analysis of AHSG gene Thr256Ser polymorphisms. Left lane path: DNA marker size 50 - 1000 bp; lanes 3, 4, and 5: CC genotype (Callele); lane 2: GG genotype (G allele); lane 1: CG genotype

The relationship between various factors with aortic calcification

Patients with diabetes (p<0.001), fetuin-A level <204 pg/mL (p<0.001), IL-6 level ≥53.05 pg/mL (p<0.001), and patients with albumin level <3.9 mg/dL (p=0.007) had higher proportion of having aortic calcification. The risk of aortic calcification increased 4.85 times (CI 95% 1.98– 11.98) if the patients with diabetes, increased 4.61 times (CI 95%: 2.49 – 8.55) in patients with Fetuin-A level <204 pg/mL, increased 12.92 times (CI 95%: 3.39– 49.21) in patients with IL-6 level ≥53.05 pg/mL, and also increased 1.41 times (CI 95%: 1.11– 1.8) in patients with albumin level of <3.9 mg/dL (Table 2).

The group of patients who had G alleles, both heterozygous and homozygous (CG and GG genotypes), was more experienced of aortic calcification (91.2%), so they have 2.03 times (CI 95%: 1.48– 2.80) higher risk of developing aortic calcification comparing to C homozygous group (CC genotype) of patients.

Table 2. The characteristic of haemodialysis (HD) patients with or without aortic calcification

Variable	No (%) of patients with aortic calcification		p	PR	95% CI
	No (n=32)	Yes (n=74)			
Age (year)			0.26	1.16	0.88 – 1.54
<60	18 (26.5)	50 (73.5)			
≥60	14 (36.80)	24 (63.2)			
Duration of HD (months)			0.35	1.13	0.87 – 1.46
< 60	15 (26.3)	42 (73.7)			
≥ 60	17 (34.70)	32 (65.3)			
Diabetes mellitus			<0.001	4.85	1.98 – 11.98
Yes	13 (15.7)	70 (84.3)			
No	19 (82.6)	4 (17.4)			
BMI Classification			0.12	1.23	0.97 – 1.57
Underweight-normal	26 (34.7)	49 (65.3)			
Overweight-obese	6 (19.4)	25 (80.6)			
Calcium (mg/dL)			0.20	1.2	0.9 –1.5
≤ 9.5	22 (34.9)	41 (65.1)			
>9.5	10 (23.3)	33 (76.7)			
Phosphate (mg/dL)			0.35	1.13	0.87 – 1.46
≤5.5	17 (34.7)	32 (65.3)			
>5.5	15 (26.3)	42 (73.7)			
Fetuin-A (pg/ml)			<0.001	4.61	2.49 – 8.55
<204	2 (2.9)	66 (97.1)			
≥204	30 (78.9)	8 (21.1)			
IL-6 (pg/ml)			<0.001	12.92	3.39 – 4921
<53.05	26 (92.9	2 (7.1)			
≥53.05	6 (7.7)	72 (92.3)			
Albumin (g/dL)			0.007	1.41	1.11 – 1.8
<3.9	6 (15.9)	37 (84.1)			
≥3.9	25 (40.3)	37 (59.7)			
Polymorphisms, n (%)			<0.001	2.03	1.48 – 2.80
CC genotype	27 (55.1)	22 (44.9)			
CG + GG genotype	5 (8.8)	52 (91.2)			

PR, Prevalence Ratio; 95%CI, Confidence Interval; BMI, body mass index

Unlike the case with the variables above, even though the statistical analysis showed an insignificant relationship (p≥0.05), patients who had a higher BMI (80.6%) and calcium level of >9.5 mg/dL (76.7%) experienced aortic calcification more compared to patients who had an underweight-normal BMI (65.3%) and calcium level of ≤ 9.5 mg/dL (65.1%). Because these variables had a p-value of <0.25, all could be included in multivariate analysis.

All patients with homozygous G allele (GG genotype) and the majority of patients with heterozygous G alleles (CG genotype) had aortic calcification, whereas more patients with homozygous C (CC genotypes) had no aortic calcification (p<0.001) (Figure 2).

The location of aortic calcification was also associated with the Fetuin-A level. Patients who

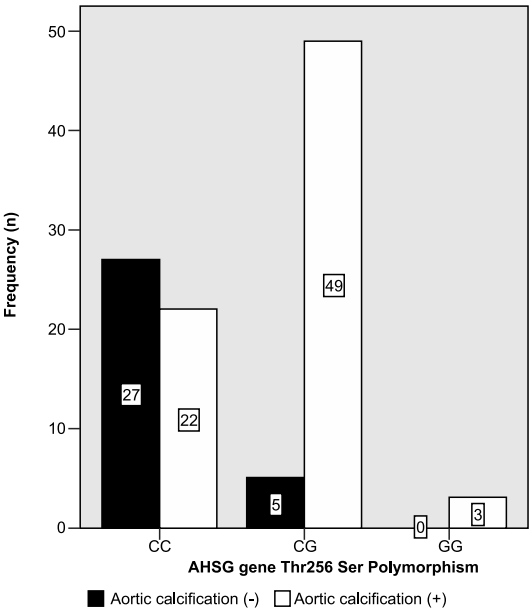

Figure 2. The association between AHSG gene Thr256Ser polymorphisms with aortic calcification

were calcified in both layers (the tunica intima and media), had the lowest mean (192.7±37.5 pg/mL) and median (199 pg/mL) of Fetuin-A level, compared to patients who were only calcified in tunica media (mean 234.1±29 pg/mL and median 238.5 pg/mL) or tunica intima alone (mean 260.4±19.9 pg/mL and median 258 pg/mL) ($p<0.001$).

A correlation test was conducted to determine the strength of the correlation between several variables with the AAC score. There was a statistically significant correlation between AAC score with Fetuin-A level ($r = -0.9$; $p<0.001$), and IL-6 level ($r=0.9$; $p<0.001$), where with the lower the level of Fetuin-A and the higher IL-6 levels of a patient, the AAC score will be higher. The value of $r=0.90$ indicates that the strength of the correlation between the two variables is strong.

Factors that influence the occurrence of vascular calcification

The purpose of this analysis was to see which variables were the most dominant ones affecting vascular calcification in regular HD patients. The variables that will be included in this multivariate test were variables with $p\leq0.25$ in the bivariate analysis that had been done before, namely diabetes, BMI, calcium level, Fetuin-A level, IL-6 level, albumin level, and AHSG gene polymorphism. Based on Table 3, a formulation can be made in predicting the occurrence of aortic calcification: aortic calcification prediction = -9.56+3.09, Fetuin-A level < 204 pg/mL+2.97, IL-6 level ≥53.05 pg/mL.

Table 3. Multivariate analysis of factors associated with vascular calcification (final step)

Risk factor	B	p	PR (CI 95%)
Fetuin-A levels < 204 pg/ml	3.09	0.001	22.0 (3.32-145.91)
IL-6 levels ≥ 53.05 mg/dL	2.97	0.002	19.50 (2.87-132.41)
Constanta	-9.56	<0.00	

Logistic regression test: B, Backward test; PR (CI95%), Prevalence Ratio (Confidence Interval);

DISCUSSION

Chronic kidney disease (CKD) is a serious health problem. It has many risk factors that lead to declining of renal function. Genetic components, gender, especially men, age, diabetes, hypertension, are some of those risk factors (11). Our study found that the prevalence of CKD was more common in male patients (61.3%).This result is in line with the research of Lumtergul et al. in CKD patients. They found that between two groups of dialysis and non-dialysis patients in Thailand, the majority of patients were male (55.4% and 53.5%, respectively) (12). In this study, we found that hypertension was the most common comorbid disease. Hypertension increases intraglomerular pressure leading to glomerulosclerosis and decreases renal function (10).

In this study, the median calcium level, mean phosphate level, and calcium phosphate multiplication were still within the target range for dialysis patients determined by Kidney Disease Outcomes Quality Initiative (K/DOQI), which is 8.4-9.5 mg/dL for calcium, 3.5-5.5 mg/dL for phosphate and <55 mg^2/dL^2 for multiplication of calcium phosphate (13). Its target range was achieved probably because almost all patients had taken the phosphate binders drugs. However, unfortunately, data about the type of phosphate binder medicines consumed by patients were not available.

Fetuin-A levels in this study were lower, whereas IL-6 levels were higher than normal values. In line with our study, El-Shehabyet al. in Egypt also found that serum Fetuin-A concentrations were lower in HD patients compared to the control group (14). Likewise, research conducted by Alwahaibi et al. in Oman found that the mean serum IL-6 concentration was higher in HD patients compared to healthy individuals (15). Uremia, genetic factors such as Single Nucleotide Polymorphism (SNPs), persistent infection, oxidative stress, excess fluid, obesity, and old age can increase IL-6 concentrations and reduce

Fetuin-A levels. Likewise, with other dialysis-related factors such as bio-compatible membranes, non-sterile dialysate, and bank filtration (7).

From the results of the AHSG gene polymorphisms, it was found that the majority of patients had CG genotype (50.9%), followed by CC genotype (46.2%) and GG genotype (2.8%). Our finding is less consistent comparing with a study conducted by Altuntas et al. in 2017 involving 152 HD patients in Turkey. They found that the majority of research subjects had CC genotype (61.8%), followed by CG genotype (34.2%), and GG genotype (4.0%) (16). This difference may be due to different races between the subjects of this study with previous studies.

This study proves that the prevalence of vascular calcification based on plain abdominal lateral radiography examination in chronic haemodialysis patients is quite high, 69.8% with a median (min-max) AAC score of 7 (0-22). Publications in recent years have shown that the prevalence of vascular calcification varies greatly from 60-100%, depending on the diagnostic method and the location of examination used, and in the region where the study was carried out (17). Vascular calcification in HD patients can occur in both arterial layers, intima, and media. Both types can be observed through the abdominal aorta (18). Our results found that most patients experienced calcification in both tunica (42.5%) followed by tunica media (22.6%).

The location of vascular calcification was related to the levels of Fetuin-A (19). The same results were also evident in this study and previous studies, which showed that the Fetuin-A level was independently associated with vascular calcification, i.e. the lower level of Fetuin-A, the more extensive vascular calcification and the more rigid aorta (19,20), so that patients who experienced vascular calcification in both tunica intima and media had the lowest level of Fetuin-A compared to other groups. Ziyrek et al. were found that a lower level of Fetuin-A had a thicker tunica intima and media (21).

Fetuin-A, also called Alpha 2-Heremans Schmid Glycoprotein (AHSG), is a cysteine protease inhibitor. From the literature, Fetuin-A has a role in inhibiting the process of osteoblastic calvaria mineralization in mouse matrix cells (22,23). Fetuin-A level is usually lower in CKD patients and has been linked to vascular calcification (3). Our results statistically show that the lower the level of Fetuin-A, the higher the risk of vascular calcification. In line with this study, Joachim et al. and Zheng et al. found that the prevalence of vascular calcification was statistically significantly more common in the lower Fetuin-A group compared to the higher Fetuin-A group (24,25). El Shehaby et al. (5) also found that low serum Fetuin A level had a significant association between high calcium score and valvular calcification, and Marechal et al. (2) showed that low serum Fetuin-A level was independently associated with aortic calcification and higher risk for cardiovascular events. Several studies have identified Fetuin-A as a connector that is lost in mediating inflammation, atherosclerosis, and calcification in CKD patients, which is known as a malnutrition-inflammatory-atherosclerosis-calcification syndrome (2).

In the prior study (6), the AHSG gene polymorphism had an impact on its transcriptional status and potentially leading to a reduction of serum Fetuin-A concentration, which has a role in inhibiting calcification. Therefore, if there is a decrease in Fetuin-A serum concentration, it may affect the development of skeletal and ectopic calcification (6). A person carrying the Thr256Ser amino acid reduced his serum Fetuin-A level significantly (26). Stenvinkel et al. (7) found that patients carrying the AHSG 256Ser allele had a lower Fetuin-A level than a patient carrying a 256Thr allele. This genotype, along with inflammation, has a higher risk of cardiovascular death, which seems to be caused by accelerated atherosclerotic and calcification due to low Fetuin-A levels in renal disease (7). Our study also had similar results. The group of patients who had G alleles, both heterozygous and homozygous (CG and GG genotypes), is more experienced in vascular calcification (91.2%), so they had a higher risk of developing vascular calcification comparing with the C homozygous group (CC genotype).

In CKD, vascular smooth muscle cells (VSMCs) changes to osteochondrogenic cells and cause the release of matrix vesicle, the formation of calcium/phosphate nanocrystal nucleation points. These processes are mediated by inflammation (27). Interleukin-6 (IL-6) is a pro-inflammatory cytokine that increases in patients with CKD (28). Increased IL-6 has shown to play an independent role in the progression of carotid atherosclerosis in the first 12 months of dialysis therapy (28). Our study has shown that patients with IL-6 levels ≥70 pg/mL had a higher risk of vascular calcification. Our study was similar to Kaminska et al. who found that IL-6 was correlated with calcium score

as a prediction of vascular calcification in CKD patients (28). Tint et al. also found that vascular calcification correlated with inflammation and was marked by high levels of IL-6 (7).

Besides inflammation, malnutrition states, which are assessed by serum albumin concentration, are also an independent risk factor of vascular calcification. A high circulating calciprotein complexes have been detected in pathological vascular calcification. Albumin prevents maturation of calciprotein particles as pathological mineralization (22,23). Fetuin-A level in haemodialysis patients shows a positive correlation with albumin serum (29). Stenvinkel et al. and Hamano et al. also found an association between Fetuin-A and albumin (2). So with a decrease in Fetuin-A levels, the possibility of albumin levels will also decrease (30). In our study, patients with albumin levels<3.9 mg/dL had a higher risk of vascular calcification. Our results were consistent with Choi et al. They found that in haemodialysis patients, the AAC progression was in line with lower levels of albumin (31).

Diabetes mellitus has a risk of increasing the incidence of coronary calcification (32). Hyperglycemia can increase the expression of vascular Bone Morphogenic Protein (BMP)-2 and BMP-4. This high activity of BMP was associated with vascular calcification (33). We found that diabetes as a comorbid disease of CKD had a significant relationship with vascular calcification. Our findings were similar to Russo et al. who found that risk vascular calcification was higher in people with diabetes compared with non-diabetics (33).

The relationship between calcium dysregulation and vascular calcification remains controversial. It is difficult to show the direct effect of long-term calcium or phosphate levels on vascular calcification because the levels of both often change and are rarely constant in HD patients (34). Previous studies have shown that there is a relationship between increased serum calcium level>10.5 mg/dL and serum phosphorus level>5.5 mg/dL with calcification in CKD patients (35). However, the presented study and the research conducted by Toussaint et al. obtained different results, in which there was no relationship between phosphate level with vascular calcification (36). This may be due to selection bias when blood sampling is only done once. Also, data on the consumption of phosphate binders drugs are unclear, so there is no known effect on calcium and phosphate levels when a blood sample is collected.

In conclusion, AHSG Thr256Ser gene polymorphism showed an association with vascular calcification in regular haemodialysis patients. The group of patients who had G allele heterozygous or homozygous (CG and GG genotypes) had a higher risk of vascular calcification. Besides, serum Fetuin-A levels and serum IL-6 levels are the most dominant risk factors of vascular calcification.

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TRANSPARENCY DECLARATION

Conflicts of interest: None to declare.

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