

Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia  
 Open Access Macedonian Journal of Medical Sciences. 2020 May 05; 8(A):185-190.  
<https://doi.org/10.3889/oamjms.2020.3520>  
 eISSN: 1857-9655  
**Category:** A - Basic Sciences  
**Section:** Genetics



# AHSG Thr256Ser Gene Polymorphism as a Predictor of Low Serum Fetuin-A Levels in Indonesian Maintenance Hemodialysis Patients

Riri Andri Muzasti<sup>1\*</sup>, Suhardjono Suhardjono<sup>1</sup>, M. B. Purwanto<sup>2</sup>, R. J. Sembiring<sup>3</sup>

<sup>1</sup>Departement of Internal Medicine, Division of Nephrology and Hypertension, Universitas Sumatera Utara, Medan, Indonesia;

<sup>2</sup>Departement of Internal Medicine, Division of Nephrology and Hypertension, Universitas Negeri Surakarta, Solo, Indonesia;

<sup>3</sup>Departement of Clinical Pathology, Universitas Sumatera Utara, Medan, Indonesia

## Abstract

**BACKGROUND:** Vascular calcification (VC) is a risk factor for cardiovascular morbidity and mortality in maintenance hemodialysis (MHD) patients. The effect of *alpha2-Heremans Schmid glycoprotein (AHSG) Thr256Ser* gene polymorphism toward serum fetuin-A levels as one of the VC inhibitors in MHD patients is still unclear.

**OBJECTIVE:** This study aims to investigate the relationship between *AHSG Thr256Ser* gene polymorphism toward serum fetuin-A levels in MHD patients in Indonesia.

**METHODS:** The research study design used was cross-sectional. Serum fetuin-A levels were assessed by enzyme-linked immunosorbent assay method, and polymerase chain reaction-restriction fragment length polymorphism determined *AHSG Thr256Ser* gene polymorphism. Multivariate linear regression analysis was used to analyze the factors related to serum fetuin-A levels.  $p < 0.05$  was considered as statistically significant.

**RESULTS:** Median value of serum fetuin-A levels was 235.0 pg/ml (ranging 78–756) with mean  $259.99 \pm 119.36$  pg/ml. Out of 106 patients involved, the distribution of *AHSG Thr256Ser* genotype frequency was 54 (50.9%) respondents had CG genotype, 46.2% had CC genotype, and the others (2.8%) had GG genotype. Patients with homozygous C allele (CC genotype) were 0.1 times more protected (CI 95%: 0.1–0.3) to have low serum fetuin-A levels, compared with patients with G allele either in a homozygote (GG genotype) or in the heterozygote (CG genotype). From the multivariate analysis results, it can be obtained a formulation in predicting serum fetuin-A level, which is: Serum fetuin-A level prediction =  $276.59 + 150.49 \text{ DM} - 0.26 \text{ serum interleukin} -6 \text{ level} - 43.58 \text{ AHSG Thr256Ser gene polymorphism}$

**CONCLUSIONS:** *AHSG Thr256Ser* gene polymorphism had a significant relationship with serum fetuin-A levels in MHD patients in Indonesia. Subjects with G allele (CG and GG genotypes) had lower serum fetuin-A levels than those with CC genotype.

**Edited by:** Sinisa Stojanoski  
**Citation:** Muzasti RA, Suhardjono S, Purwanto MB, Sembiring RJ. *AHSG Thr256Ser* Gene Polymorphism as a Predictor of Low Serum Fetuin-A Levels in Indonesian Maintenance Hemodialysis Patients. Open Access Maced J Med Sci. 2020 May 05; 8(A):185-190. <https://doi.org/10.3889/oamjms.2020.3520>

**Keywords:** Alpha 2-Heremans Schmid glycoprotein Thr256Ser gene polymorphism; Fetuin-A; Hemodialysis

**\*Correspondence:** Riri Andri Muzasti, Department of Internal Medicine, Faculty of Medicine, Universitas Sumatera Utara, dr. Mansyur 5 Medan, Indonesia. Phone: +6281260556872. E-mail: riri.andri@usu.ac.id

**Received:** 13-Aug-2019

**Revised:** 11-Mar-2020

**Accepted:** 20-Apr-2020

**Copyright:** © 2020 Riri Andri Muzasti, Suhardjono Suhardjono, M. B. Purwanto, R. J. Sembiring

**Funding:** This research did not receive any financial support

**Competing Interest:** The authors have declared that no competing interest exists

**Open Access:** This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)

## Introduction

Cardiovascular disease (CVD) is found in >50% of patients undergoing dialysis. This disease is the primary cause of morbidity and mortality in end-stage renal disease (ESRD) patients undergoing maintenance hemodialysis (MHD), where the hazard ratio for death caused by this CVD is 20 times higher than in general populations [1].

One of the cardiovascular risk factors in HD patients is vascular calcification (VC) [1]. There are two types of VC in patients with chronic kidney disease (CKD), which include intima calcification and medial calcification (Monckenberg's medial sclerosis). Both of these calcifications have different complications. Intima calcification is related to atherosclerotic plaque, causing blood vessel occlusion; on the other hand, medial calcification provokes blood vessel stiffness [2].

The pathogenesis of VC is complex and is not fully understood yet [2]. *In vitro* study showed that

VC process is not just simple deposition of calcium phosphate crystals, but is a process similar to bone osteogenesis. This process involves vascular smooth muscle cells (VSMCs) transdifferentiation to cells resembling bone progenitor cells [1]. VC process is an active process but can be modified, although VSMCs gradually experience apoptosis and vesicle formation changing VSMCs phenotype into osteoblast cells that can undergo mineralization process. Several VC inhibitors have been investigated, such as matrix GLA protein, osteoprotegerin, pyrophosphates, and fetuin-A [2].

Fetuin-A also is known as alpha 2-Heremans Schmid glycoprotein (AHSG) is a part of the cystatin family from cysteine protease inhibitors. Fetuin-A interacts with the small mineral core to form dissolved colloid particles so that basic calcium phosphate (BCP) deposition does not happen and works as a calcification inhibitor in a concentrated extracellular environment. As a result, fetuin-A can prevent calcification in circulation without intervening normal bone mineralization.

Furthermore, in the cellular level, fetuin-A inhibits apoptosis from VSMCs and helps binding apoptosis bodies with surrounding cells aiming toward cleaning and thus lowering potential to form BCP [2].

In CKD patients, serum fetuin-A levels are lower than healthy control subjects [2]. Three *fetuin-A* genotypes can probably be seen, which are Thr/Thr (C allele), Thr/Ser (heterozygous), and Ser/Ser (G allele). In several kinds of literature, it is said that there is a relationship between serum levels and gene polymorphism coding fetuin-A [3]. Stevinkel *et al.*, states that Dialysis patients in Sweden showed lower serum fetuin-A levels in fetuin-A Thr256Ser [4]. As well as Axelsson *et al.* concluded that *fetuin-A* (C→G), *Thr256Ser* gene polymorphism affected circulation serum fetuin-A levels. On the contrary, another study found that *fetuin-A* (C→G) distribution, gene polymorphism did not affect serum fetuin-A levels [2]. Due to this contradiction, this research was conducted to determine whether there is a relationship between *AHSG Thr256Ser* gene polymorphism toward serum fetuin-A levels in regular HD patients in Indonesia.

## Methods

### Study design

This study was a cross-sectional study involving 106 regular hemodialysis (HD) patients in the HD unit in Rumah Sakit Khusus Ginjal Rasyida. This study was conducted from January to May 2018. Patients included in this study were ≥18 years old, undergoing HD ≥30 months, and were willing to participate by signing informed consent. Patients with liver diseases, active infections, and malignancy were excluded from the study. Helsinki's Declaration was conducted in this study.

### Assessments

Personal information and medical history (such as age, gender, race, smoking history, DM, hypertension, CVD, malignancy, and duration of HD) were obtained through medical records and interviews. Calculation of body mass index (BMI) was collected by measuring weight in kilograms divided by height in meter square. Consequently, blood drawing, routine, and particular tests were performed. Routine tests included calcium, phosphate, and albumin. Specific tests performed were quantitative fetuin-A test and DNA extraction as well as functional *fetuin-A* genotype polymorphism test. Five milliliters venous blood would be drawn from each patient in the morning after the patient fasted at night before blood was drawn. Five hundred micro whole blood would be separated in

an Eppendorf containing ethylenediaminetetraacetic acid 5% and was kept in -20° for Thr256Ser testing using polymerase chain reaction (PCR) technique. The remaining samples would go into disposable tubes and were left to clot, then were put into centrifuge machine at 1200 rpm for 10–15 min to separate serum. Serum was divided into three aliquots and was frozen. Serum fetuin-A quantitative and interleukin-6 (IL-6) testing were assessed by enzyme-linked immunosorbent assay (ELISA) method.

### Statistical analysis

Research data were analyzed using Statistical Package for the Social Sciences version program (SPSS program version 22.0 SPSS Inc., Chicago, IL, USA). Normality test by Kolmogorov–Smirnov was put into use. Numeric data were displayed as median and range as mean±standard deviation. Categorical data were demonstrated in n (percent). To compare serum fetuin-A levels with patient's characteristics, the Mann–Whitney U-test was used. If data are coming from more than three groups/populations, then the Kruskal–Wallis test was put into use. To see the comparison of HD patients characteristics based on serum fetuin-A levels, Chi-square test was used for categorical-categorical variables and Mann–Whitney U-test for categorical-numerical variables. If expected count value <5, as a result, Fisher's exact test was adapted. Correlation between variables was assessed with Spearman correlation or Pearson's coefficient correlation. p-value was said to be significant if < 0.05 (p < 0.05).

## Results

### Samples characteristics

Out of 106 respondents, 65 subjects (61.3%) were male and the remaining 41 subjects (38.7%) were female. Patients aged between 21 and 78 years old with 53.89 ± 11.44 years old. Patients on average underwent HD for the 1<sup>st</sup> time at age 48.19 ± 12.79 years old. Most patients went through HD for 10 h in 1 week (73.6%) and typically had been going through HD for 69.44 ± 34.57 months (range 34–237). Average respondents' BMI was overweight (24.22 ± 4.09 kg/m<sup>2</sup>).

Through ELISA method, median serum fetuin-A levels and IL-6 were obtained which were 235.0 pg/ml (range 78–756) and 70.7 pg/ml (range 25.4–898.0) with mean 259.99 ± 119.36 pg/ml and 99.64 ± 115.51 pg/ml. On the other hand, *AHSG Thr256Ser* genotype frequency was collected by PCR-restriction fragment length polymorphism test with the result such as 54 (50.9%) respondents had CG genotypes, 46.2% of respondents had CC genotypes, and the remaining (2.8%) had GG genotypes.

**The relationship between various factors with serum fetuin-A levels**

The comparison of serum fetuin-A levels based on HD patients characteristics is shown in Table 1. Statistical analysis significantly showed that patients with DM had a higher mean (407.6 ± 176.6 pg/ml) and higher median (331 pg/ml) serum fetuin-A levels than in patients without DM (p < 0.001). As well as, patients whose serum IL-6 <70.7 pg/ml and albumin ≥3.9 mg/dL had higher mean and median serum fetuin-A levels compared with patients with serum IL-6 ≥70.7 pg/ml and albumin <3.9 mg/dL (p < 0.001 and p = 0.03).

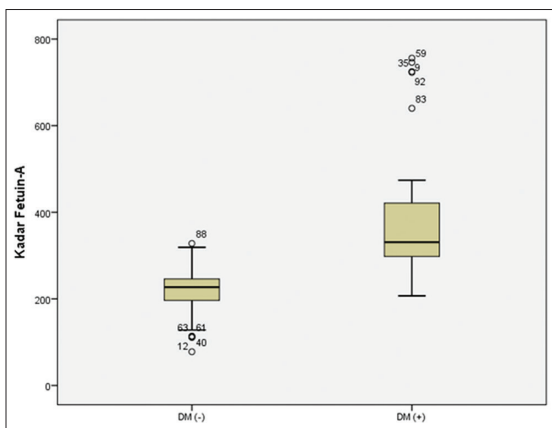
**Table 1: Comparison of serum fetuin-A levels based on HD patients characteristics**

Variables	Serum fetuin-A levels		p
	Mean ± SD	Median (range)	
Age (years)			
<60	260.3 ± 128.5	232.5 (78–756)	0.25 <sup>#</sup>
≥60	259.4 ± 102.5	237.5 (113–725)	
HD vintage (months)			
<60	265.9 ± 129.3	231 (113–756)	0.52 <sup>#</sup>
≥60	253.1 ± 107.6	242 (78–723)	
DM			
No	219.1 ± 45.5	227 (78–328)	<0.001 <sup>#</sup>
Yes	407.6 ± 176.6	331 (207–756)	
Klasifikasi IMT			
Underweight – normal	266.0 ± 120	238 (111–746)	0.28 <sup>#</sup>
Overweight – obese	245.3 ± 117	229 (78–756)	
Serum calcium (mg/dL)			
≤9.5	268.3 ± 152.9	227 (111–746)	0.33 <sup>#</sup>
>9.5	254.3 ± 90.5	238 (78–756)	
Serum phosphate (mg/dL)			
≤5.5	251.5 ± 105.1	233 (111–725)	0.69 <sup>#</sup>
>5.5	269.9 ± 134.5	235 (78–756)	
Serum IL-6 (pg/ml)			
<70.7	322.4 ± 138.8	270.0 (191–756)	<0.001 <sup>#</sup>
≥70.7	197.6 ± 39.4	208.0 (78–253)	
Serum albumin (mg/dL)			
<3.9	232.4 ± 97.2	228.0 (78–723)	0.03 <sup>#</sup>
≥3.9	279.6 ± 130.1	241.5 (112–756)	
Polymorphism			
CC genotype	319.1 ± 147.8	266.0 (184–756)	<0.001 <sup>*</sup>
CG genotype	214.1 ± 43.5	216.5 (111–328)	
GG genotype	120.3 ± 4.5	113.0 (78–170)	

<sup>#</sup>Mann-Whitney U-test, <sup>\*</sup>Kruskal-Wallis test. HD: Hemodialysis, IL: Interleukin.

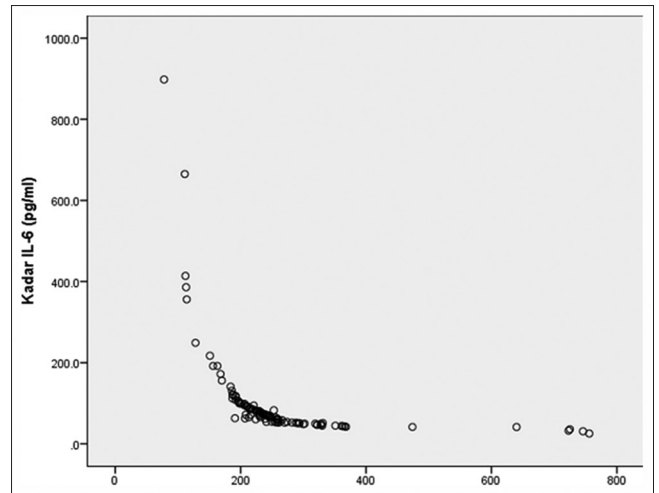
Statistical test significantly showed that patients with CC genotypes had the highest mean and median serum fetuin-A levels, while patients with GG genotypes had the lowest mean and median serum fetuin-A levels (p < 0.001) compared with the other AHS gene genotypes.

Figure 1 shows that patients with DM had higher mean and median serum fetuin-A levels compared with patients without DM (p < 0.001).



**Figure 1: The comparison of serum fetuin-A levels based on DM comorbidity**

To determine the power correlation between serum IL-6 level and serum fetuin-A level, Spearman's rho correlation test was put into test. Figure 2 shows that serum fetuin-A had a significant negative correlation with serum IL-6 level, which means that the higher serum IL-6 level, the lower serum fetuin-A level. R = 0.9 showed that the power correlation between two variables was powerful.



**Figure 2: Correlation between serum interleukin -6 level and serum fetuin-A level**

HD patient characteristics based on serum fetuin-A level are shown in Table 2. Statistical test significantly showed that group of patients with low serum fetuin-A level was dominated by patients without DM (60.2%) (p < 0.001), had serum IL-6 ≥70.7 pg/ml (86.8%) (p < 0.001) and serum albumin <3.9 mg/dL (61.4%) (p = 0.03). Furthermore, based on its AHS gene polymorphism, it can be seen that more patients with GG genotypes had lower serum

**Table 2: Characteristics of MHD patients based on serum fetuin-A levels**

Variables	Serum fetuin-A levels		p	PR	95% CI
	Low (n=52)	High (n=54)			
Age (years)					
<60	35 (51.5)	33 (48.5)	0.51 <sup>*</sup>	1.2	0.8–1.8
≥60	17 (44.7)	21 (55.3)			
HD vintage (months)					
<60	30 (52.6)	27 (47.4)	0.43 <sup>*</sup>	1.3	0.9–1.9
≥60	22 (44.9)	27 (55.1)			
DM					
No	50 (60.2)	33 (39.8)	<0.001 <sup>*</sup>	7.0	1.8–26.4
Yes	2 (8.7)	21 (91.3)			
IMT (kg/m <sup>2</sup> )					
Underweight – normal	35 (46.7)	40 (53.3)	0.44 <sup>*</sup>	1.2	0.8–1.8
Overweight – obese	17 (54.8)	14 (45.2)			
Serum calcium (mg/dL)					
≤9.5	23 (43.4)	30 (56.6)	0.24 <sup>*</sup>	1.6	0.7–3.4
>9.5	29 (54.7)	24 (45.3)			
Serum phosphate (mg/dL)					
≤5.5	29 (50.9)	28 (49.1)	0.53 <sup>*</sup>	1.1	0.7–1.6
>5.5	23 (46.9)	26 (53.1)			
Serum IL-6 (pg/ml)					
<70.7	6 (11.3)	47 (88.7)	<0.001 <sup>*</sup>	6.7	3.3–13.5
≥70.7	46 (86.8)	7 (13.2)			
Serum albumin (mg/dL)					
<3.9	27 (61.4)	17 (38.6)	0.03 <sup>*</sup>	2.4	1.1–5.2
≥3.9	25 (40.3)	37 (59.7)			
Polymorphism, n (%)					
CC genotype	12 (24.5)	37 (75.55)	<0.001 <sup>*</sup>	0.1	0.1–0.3
CG genotype	37 (68.5)	17 (31.5)			
GG genotype	3 (100.0)	0 (0.0)			
CC genotype	12 (24.5)	37 (75.55)			
CG+GG genotype	40 (70.2)	17 (29.8)			

<sup>\*</sup>Chi-square, <sup>#</sup>Mann-Whitney U, <sup>\*</sup>Fisher's exact. MHD: Maintenance hemodialysis, HD: Hemodialysis, IL: Interleukin.

fetuin-A levels compared with the other groups of subjects ( $p < 0.001$ ).

From this table, it can also be concluded that lower serum fetuin-A levels were 7.0 times (CI 95%: 1.8–26.4) more likely occurred in patients without DM, 6.7 times (CI 95%: 3.3–13.5) in patients with serum IL-6  $\geq 70.7$  pg/ml and 2.4 times (CI 95%: 1.1–5.2) in patients with serum albumin  $< 3.9$  mg/dL. Patients who had homozygous C allele (CC genotype) were 0.1 times (CI 95%: 0.1–0.3) more protected to have low serum fetuin-A level, compared with patients who had either homozygous (GG genotype) or heterozygous (CG genotype) G allele.

Figure 3 showed that an individual with GG genotype was correlated with low serum fetuin-A levels and high serum IL-6 levels. In other words, there is an inverse relationship between serum IL-6 level and serum fetuin-A level in every *AHSG* gene genotype.

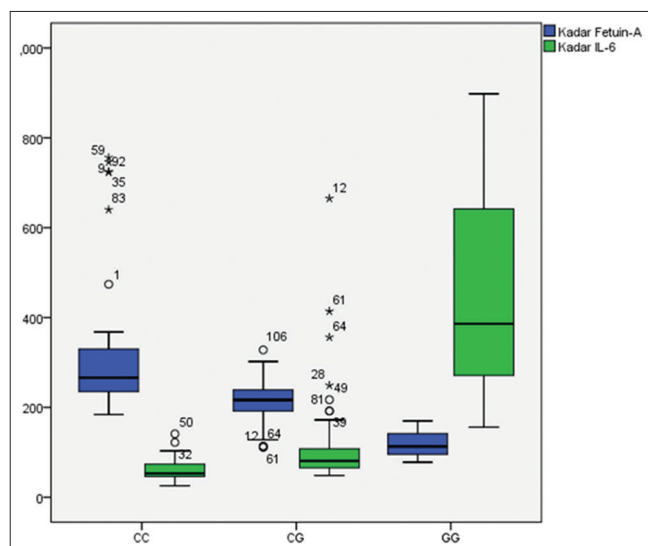


Figure 3: The effect of alpha 2-Heremans Schmid glycoprotein *Thr256Ser* gene polymorphism toward serum fetuin-A and IL-6 levels

To determine the variables affecting serum fetuin-A levels significantly, multivariate analysis on variables was performed with  $p \leq 0.25$  in a previously performed bivariate test. Variables that were included in the multivariate test include age when first undergoing HD, duration of HD, DM, serum IL-6, albumin, and *AHSG* gene polymorphism.

According to the statistical test, it was concluded that DM ( $p < 0.001$ ), serum IL-6 ( $p < 0.001$ ), and *Thr256Ser* polymorphism ( $p = 0.001$ ) were significant factors related to serum fetuin-A levels. Out of that table, a formula can be created in predicting serum fetuin-A levels as follows:

Predicted serum fetuin-A level =  $320.49 + 150.49 \text{ DM} - 0.26 \text{ serum IL-6 level} - 43.58 \text{ polymorphism}$

Slope value for DM variable was +150.49, which means that every increase of one unit DM increased serum fetuin-A as much as 150.49. An increase of one unit implied that there was an alternation from without

Table 3: Multivariate analysis of factors related to serum fetuin-A levels at the final step

Model	Variables	Adjusted R2	B	p		
1	Intercepts	52.4%	369.41	0.003		
	DM		149.63	<0.001		
	Serum IL-6		-0.28	<0.001		
	Serum albumin		-20.20	0.45		
	Polymorphism		-46.46	0.01		
	HD duration		0.38	0.11		
	Age		-0.68	0.35		
2	Intercepts	52.6%	283.70	<0.001		
	DM		149.52	<0.001		
	Serum IL-6		-0.26	0.001		
	Polymorphism		-45.05	0.01		
	HD duration		0.37	0.12		
	Age		-0.59	0.41		
	3		Intercepts	52.7%	252.75	<0.001
DM		148.81	<0.001			
Serum IL-6		-0.26	0.001			
Polymorphism		-44.01	0.02			
HD duration		0.35	0.14			
4		Intercepts	52.1%		320.49	<0.001
		DM			150.49	<0.001
	Serum IL-6 $\geq 70.7$ pg/ml	-0.26		0.001		
	Polymorphism	-43.58		0.02		

\*Linear regression test, HD: Hemodialysis, IL: Interleukin.

DM into with DM. On the other hand, slope value for polymorphism was -43.58, which means for every increase of one unit polymorphism lowered serum fetuin-A levels as much as 43.58. It is meant as one unit increased was changes from C allele (CC genotype) becoming G allele (CG+GG genotype).

## Discussion

CKD is related to the increased risk of CVD. ESRD patients with dialysis have death risk 10–20 times higher caused by CVD complications compared with the general populations. VC is a strong independent risk factor contributing to mortality caused by CVD. VC can cause arterial stiffness, an increase in blood pressure, left ventricle hypertrophy, and death as a result of CVD [2].

Blood vessel changes observed in CKD patients who are not only atherosclerosis but also wide arteriosclerosis related to VC on both media and intima. Blood vessel stiffness and the extent of calcification are considered as prognosis predictor of death in HD patients. This extensive calcification is considered linked to the decrease in calcification inhibitor or blood vessel protection factor such as fetuin-A [3]. In CKD patients, serum fetuin-A levels are significantly lower than in control subjects [2].

Fetuin-A is a glycoprotein weighing 64-kDa that has a relatively high concentration in human's serum (300–1000  $\mu\text{g/ml}$ ). Fetuin-A plays a role as VC inhibitor. As a result, it can inhibit calcium-phosphate deposition. Fetuin-A inhibits ectopic calcification of mineral by obstructing the formation of apatite to prevent mineral precipitation [2].

This study observed the relationship between fetuin-A and its gene in patients undergoing HD.

A higher number of patients with GG genotypes (91.2%) had lower serum fetuin-A levels compared with the other group of subjects ( $p < 0.001$ ). Group of patients with CC genotype had the highest mean and median serum fetuin-A levels. Maharem *et al.* attempted to understand the pathogenesis of VC increased risk by analyzing *Thr256Ser* gene polymorphism in CKD patients with conservative therapy, HD and those who had undergone kidney transplantation and had them compared with healthy control subjects. They did not find any difference between the three fetuin-A genotypes (C→G) in all groups. However, fetuin-A distribution (C→G); *Thr256Ser* gene polymorphism showed a significant relation with how low the serum fetuin-A levels were, where subjects with CG and GG genotypes had lower serum fetuin levels than patients with CC genotype [2]. Axelson *et al.* displayed the same result. They found that circulating serum fetuin-A level was affected by *fetuin-A Thr256Ser* gene polymorphism (C-G) [5]. On the contrary, Cozzolino *et al.*, in Italy, and Zeidan *et al.* concluded that both in HD patients and control group, fetuin-A gene distribution did not show correlation with serum fetuin-A level [6], [7].

To understand serum fetuin-A genetic control, Jensen *et al.* conducted genome-wide association meta-analysis research on six populations, as part of Cohorts for Heart and Aging Research in Genomic Epidemiology consortium. *AHSG* expression is controlled by several transcriptional factors (TFs) including C/EBP- $\beta$ , NF-1, HNF-3 $\beta$ , AP-1, and ER  $\alpha$ . *SNP rs2248690*, which is located on the promoter, has a strong association with serum fetuin-A level, modifies *AHSG* transcription by changing AP-1 affinity. Although located at the end of *AHSG* region, this variant undergoes linkage disequilibrium with SNPs 4917 and rs4918 located on exon ( $r^2 > 0.80$  CEU) that has the potential to disrupt DNA chains on some TFs, including AP-1 and ER $\alpha$ . A transcription complex consisting of ER $\alpha$  and AP-1 can contribute to *AHSG* transcription induced by estrogen.

On the other hand, controlling function coming from a coding location that saves rs4917 and rs4918 needs further studies. They get rs4917 and rs4918 as missense variant located at the two last exons from the *AHSG* gene (each exon 6 and 7 has the same effect on the difference of serum fetuin-A level in the plasma). This study concluded that a genetic variant on the *AHSG* gene is strongly associated with serum fetuin-A levels. However, it cannot be excluded that there is a chance of other genetic loci playing a role in determining the fetuin-A concentration [8].

The underlying and mechanism of low serum fetuin-A levels in GG genotype compared within CC genotype have not been fully understood. The difference in results in several studies before can be explained by variation in the race and the effect of fetuin-A nucleotide polymorphism toward its serum level [2].

In this research, there were a higher mean and median serum fetuin-A levels in patients with DM than in patients without DM. Statistical test significantly showed that patients without DM had 7 times more risks (CI 95 %: 1.8–26.4) to have lower serum fetuin A levels. Verdujin *et al.* followed 1043 dialysis patients with *Thr256Ser* genotype polymorphism (rs4918) for 5 years, then measured serum fetuin-A levels in 549 patients and concluded that one of the risk factors for low serum fetuin-A levels was diabetes and inflammation [9]. Maharem *et al.* also obtained the same result, where patients with DM had higher mean and median serum fetuin-A level than in patients without DM ( $p < 0.001$ ) [2]. Srinivas *et al.* first proved 20 years ago that fetuin-A was linked to insulin receptor at tyrosine kinases. The liver and adipose tissue secrete fetuin-A. Serum fetuin-A levels correlate with various metabolic parameters, such as insulin sensitivity, glucose tolerance, lipid level, and pro- and anti-inflammatory protein levels [10]. *AHSG* gene polymorphism has been associated with DM type 2 prevalence. *AHSG* gene is located at chromosome 3q27, which has been identified as loci region susceptible to metabolic syndrome. Fetuin-A inhibits tyrosine kinase receptor activity in skeletal muscle and hepatocytes, and thus, it inhibits insulin signal transduction. As a result, insulin resistance occurs in target tissues. Because of that, in general population, fetuin-A correlates with insulin resistance, metabolic syndrome and is an independent risk factor toward DM type 2 [2], [11], [12].

Nonetheless, in the uremic environment, how the metabolic syndrome is linked to increased serum fetuin-A level which is far more complex. Although genetically, Mendelian random observational study predicted that serum fetuin-A level did not have an association with diabetes prevalence, diabetes incidence, or fasting glucose. However, the limitation of this study was that it did not count non-esterified fatty acid level, where recently it showed interaction with fetuin-A to predict the degree of insulin resistance [10].

The presence of CC genotype in an individual will increase serum fetuin-A level and decrease serum IL-6 level. There is a contradictory relation between serum IL-6 and serum fetuin-A levels in every *AHSG* gene genotype. To determine correlation power between serum IL-6 and serum fetuin-A, a correlation test was put into use. It significantly showed that the higher the serum IL-6 level was then, the lower the serum fetuin-A level was  $r = -0.9$ ,  $p < 0.001$ . Inflammation is an integral component of atherosclerosis that can worsen damage by decreasing fetuin-A synthesis and other calcification inhibitors factors [2]. Lebreton *et al.* proved that fetuin-A expression arranged opposite by pro-inflammatory cytokines such as TNF- $\alpha$ , IL-1, and IL-6, because of that fetuin-A is classified as negative acute-phase protein. The serum concentration lowers during acute inflammation response and becomes normal when the infection is successfully handled. Memoli *et al.* reported

that IL-6 significantly induced suppression of fetuin-A expression in human's hepatocytes [11].

Serum fetuin-A levels in HD patients showed a positive correlation with serum albumin [13]. Stavinkel *et al.* and Hamano *et al.* also discovered the relationship between fetuin-A and albumin [2]. In accordance with the previous research, bivariate test showed that research subjects who had serum albumin  $\geq 3.9$  mg/dL had higher mean ( $279.6 \pm 130.1$  pg/ml) and median (241.5 pg/ml) serum fetuin-A levels compared with patients who had serum albumin  $< 3.9$  mg/dL ( $p = 0.03$ ). However, on the multivariate test, this result was not consistent anymore. Hypoalbuminemia that has a strong association with fetuin-A deficiency shows involvement of malnutrition-inflammation-atherosclerosis syndrome in fetuin-A deficiency [14]. Either albumin or fetuin-A, both produced in the liver, is an important protein that has various functions. This protein decreases when there are inflammation and malnutrition [15].

## Conclusions

Fetuin-A plays an essential role in inhibiting calcification, especially in CKD patients. This research proved that *AHSG Thr256Ser* gene polymorphism had a significant association with serum fetuin-A levels in regular HD patients in Indonesia. Subjects with G allele (CG and GG genotype) had lower serum fetuin level compared with patients with CC genotype. The underlying and mechanism of low fetuin levels in GG genotype compared with CC genotype have not been fully understood yet. This can be caused by the difference in race and the effect or other fetuin-A nucleotide polymorphisms which were not analyzed in this study.

## References

- Cozzolino M, Mangano M, Stucchi A. Cardiovascular disease in dialysis patients. *Nephrol Dial Transplant*. 2018;33 Suppl 3:iii28-34. <https://doi.org/10.1093/ndt/gfy174>  
PMid:30281132
- Maharem DA, Gomaa SH, Gandhi MK, Mohamed EI. Association of serum fetuin-a and fetuin-a gene polymorphism in relation to mineral and bone disorders in patients with chronic kidney disease. *Egypt J Med Hum Genet*. 2013;14(4):337-52. <https://doi.org/10.1016/j.ejmhg.2013.07.003>
- Altuntas A, Yigit A, Uz E, Inal S. The relationship between fetuin levels and fetuin gene polymorphism in a hemodialysis patient. *Biomed India*. 2017;28(2):495-5. <https://doi.org/10.1093/ndt/gfv199.29>
- Stenvinkel P, Wang K, Qureshi AR, Axelsson J, Pecoits-Filho R, Gao P, *et al.* Low fetuin-a levels are associated with cardiovascular death: Impact of variations in the gene encoding fetuin. *Kidney Int*. 2005;67(6):2383-92. <https://doi.org/10.1111/j.1523-1755.2005.00345.x>  
PMid:15882283
- Axelsson J, Wang X, Ketteler M, Qureshi AR, Heimbürger O, Bárány P. Is fetuin-a/alpha2-heremans-schmid glycoprotein associated with the metabolic syndrome in patients with chronic kidney disease? *Am J Nephrol*. 2008;28(4):669-76. <https://doi.org/10.1159/000121358>  
PMid:18337634
- Cozzolino M, Dusso AS, Slatopolsky E. Role of calcium-phosphate product and bone-associated proteins on vascular calcification in renal failure. *J Am Soc Nephrol*. 2001;12(11):2511-6.  
PMid:11675430
- Zeidan MA, Sharara GM, Suliman HS, Zeid MM, Zytoun SS, Nomeir HM. Visfatin and fetuin-a: Novel markers for endothelial dysfunction in chronic kidney disease. *Life Sci J*. 2012;9(2):227-43.
- Jensen KM, Jensen RA, Mukamal KJ. Detection of genetic loci associated with plasma fetuin-a: A meta-analysis of genome-wide association studies from the CHARGE consortium. *Hum Mol Genet*. 2017;26(11):2156-63.  
PMid:28379451
- Verduijn M, Prein RA, Stenvinkel P, Carrero JJ, le Cessie S, Witasp A, *et al.* Is fetuin-A a mortality risk factor in dialysis patients or a mere risk marker? A Mendelian randomization approach. *Nephrol Dial Transplant*. 2011;26(1):239-45. <https://doi.org/10.1093/ndt/gfq402>  
PMid:20605840
- Trepanowski JF, Mey J, Varady KA. Fetuin-a: A novel link between obesity and related complications. *Int J Obes (Lond)*. 2015;39(5):734-41. <https://doi.org/10.1038/ijo.2014.203>  
PMid:25468829
- Celebi G, Genc H, Gurel H, Sertoglu E, Kara M, Tapan S, *et al.* The relationship of circulating fetuin-a with liver histology and biomarkers of systemic inflammation in nondiabetic subjects with nonalcoholic fatty liver disease. *Saudi J Gastroenterol*. 2015;21(3):139-45. <https://doi.org/10.4103/1319-3767.157556>  
PMid:26021772
- Sindhu S. Fetuin an (AHSG) in metabolic and inflammatory diseases: A foe or a friend. *Diabetes Obes Int J*. 2015;1(5):127. <https://doi.org/10.23880/doi-16000127>
- Oikawa O, Higuchi T, Yamazaki T. Evaluation of serum fetuin-a relationships with biochemical parameters in patients on hemodialysis. *Clin Exp Nephrol*. 2007;11(4):304-8. <https://doi.org/10.1007/s10157-007-0499-y>  
PMid:18085392
- Ann A, Makkar V, Mann S, Ghamija P, Soundrajan P. Fetuin-a and vascular calcification in Indian end-stage renal disease population. *Indian J Nephrol*. 2016;26(1):33-8. <https://doi.org/10.4103/0971-4065.157007>  
PMid:26937076
- Yamada S, Tokumoto M, Tsuruya K. Fetuin-a induced by low protein diet enhances vascular calcification in uremic rats with hyperphosphatemia. *Am J Physiol Renal Physiol*. 2015;309(8):F744-54. <https://doi.org/10.1152/ajprenal.00017.2015>  
PMid:26180236

Author Queries???

AQ6:Kindly cite table 3in text part

AQ7:Kindly check and confirm the table format.

AQ8:Kindly cite significant value