ORIGINAL ARTICLE

Fetuin-A Thr256Ser gene polymorphism as a mortality predictor in patients with chronic kidney disease on maintenance haemodialysis 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 To investigate an impact of Fetuin-A Thr256Ser gene polymorphism on the mortality rate of chronic kidney disease on maintenance haemodialysis patients in Indonesia.

Methods This is an analytic-longitudinal observational study using survival analysis with nine-month follow up on 106 maintenance haemodialysis patients. The PCR-RFLP is used to determine Fetuin-A Thr256Ser gene polymorphism and Fetuin-A serum level measured by using ELISA methods. We use time-independent cox regression analysis to investigate factors that contribute to patient survival.

Results The mean survival time of this study is 8.49±1.53 months, with a median survival of 9 months (range 1-9 months). Among 12 (11.3%) deceased patients, most of them carried GG genotype with 8.87 times risk of mortality compared to those with CC+CG genotype (p=0.005). The group of patients with IL-6 level ≥86.9 pg/mL had higher mortality with 3.64 times greater risk compared to those with IL-6 level, <86.9 pg/mL (p=0.03).

Conclusion This study revealed a significant dominance independent impact of the Fetuin-A Thr256Ser gene polymorphism on the survival rate of maintenance haemodialysis patients. These results suggest that genotype variation of Fetuin-A gene could be a potential marker to identify high mortality risk in Indonesia's maintenance haemodialysis patients, especially in Medan.

Key words: alpha-2-HS-glycoprotein, haemodialysis, mortality

Corresponding author:
Riri AndriMuzasti
Division of Nephrology and Hypertension, Department of Internal Medicine,School of Medicine, Universitas Sumatera Utara
Dr. Mansyur 5 Medan, Indonesia
Phone: +62 81 260 556 872;
Fax: +62 8161 821 6264;
E-mail: riri.andri@usu.ac.id
ORCID ID: https://orcid.org/0000-0001-7834-0740

Original submission:
27 September 2019;
Accepted:
22 November 2019
doi: 10.17392/1085-20

Med Glas (Zenica) 2020; 17(1):22-29

INTRODUCTION

The mortality of patients on haemodialysis is 30 times higher compared to the general population. The prevalence and extent of vascular calcification is a strong predictor for cardiovascular disease and all-cause mortality in chronic kidney disease (CKD) patients on maintenance haemodialysis. The mechanism remains unclear, but recently, the concern is focused on the potential role of Fetuin-A serum (1).

Fetuin-A is a glycoprotein coded by the Fetuin-A gene. The human Fetuin-A gene lies in the 3q27 chromosome. The AHSG molecule has three structural domains, i.e. two cystatin-like domains in a tandem arrangement that are encoded by three exons and unique carboxyl-terminal domain encoded by the last exon (2). Fetuin-A gene SNP may be in promoter, intron or exon. The SNP rs4918 is located on the seventh exon and functions as a code for the Thr256Ser amino acid. This gene is the most common SNP analyzed for their relation with vascular calcification (3).

High Fetuin-A plasma concentration is related to metabolic syndrome and atherogenic lipid profile. Meanwhile, a lower level of Fetuin-A is associated with inflammation and vascular calcification (4). A study by Ketteler et al. on 312 chronic haemodialysis patients revealed that patients with a low level of serum Fetuin-A show worse prognosis compared to those with a normal-high level of serum Fetuin-A (5). Mehrotra suggests that a low level of serum Fetuin-A is most likely due to an inflammatory process caused by uremia. Because of this reason, Fetuin-A is considered as a negative acute phase reactant (6).

The magnitude reduction of Fetuin-A level in inflammation is also affected by genetic predisposition. A study by Stenvinkel et al. revealed that patients who carried 256Ser allele had lower Fetuin-A level compared to those with 256Thr allele, concluding that Fetuin-A Thr256Ser genetic variation has a significant impact on mortality and lower level of Fetuin-A level (7). On the other hand, Verduijn et al. proved that Thr-256Ser gene polymorphism only had less effect on mortality risk in end-stage renal disease (8). Due to this contradiction, we are interested in investigating the impact of Fetuin-A Thr256Ser gene polymorphism on the survival rate of maintenance haemodialysis patients in Indonesia.

METHODS

Patients and study design

In this prospective study, nine months of follow up on 106 patients who underwent maintenance haemodialysis in Rasyida Renal Special Hospital (Medan, Indonesia) were analyzed using a specific study design for survival analysis. Patients with age of ≥18 years on maintenance haemodialysis for at least 30 months, who agreed to undergo a blood test and follow up, were included in this study. The patients with a loss to follow up or with emergency and hospitalization were excluded from this study.

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

Methods

The patients' medical records and interviews were used to obtain data of age, haemodialysis duration, and history of previous illness. Body Mass Index (BMI) was calculated by dividing weight in kilograms by the square of the height in meters. The Interleukin-6 level was measured using the turbidimetry method (Architect Multiagent CRP Harmonization 6K26-30, Indonesia). Calcium was performed by measuring serum level based on the cresol phthalein complex one method (ADVIA 03932883 RevA, Indonesia), phosphate was performed by measuring level based on the phosphomolybdate method (ADVIA 024220623 Reva, Indonesia), while albumin level 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 enzyme-linked immunosorbent assay (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 ThermoScientific(Thermo Fisher Scientific, USA). The PCR-RFLP technique was used to detect the Thr256Ser gene.

Statistical analysis

Data distribution was tested for normality by using the Kolmogorov Smirnov test. Numerical data were represented in mean±SD or median depended on its distribution, while categorical data were represented in numbers (percentages). If the variable was categorical and numerical, the T-test independent or Mann Whitney U test was used. In the case of two categorical variables, the $\chi 2$ test was used. The impact of various characteristics on mortality was analyzed with Kaplan-Meier Survival Curves. The relative risk of mortality was determined with univariate and multivariate cox regression analysis. The $p < 0.05$ was considered statistically significant.

RESULTS

From 106 patients, 65 (61.3%) were male, with the age range 21-78 years (the mean of 53.89 ± 11.44). Most of the patients have been on haemodialysis for 69.44 ± 34.57 months (range 34-237). Hypertension was the most common comorbidity, 76 (71.7%), followed by diabetes, cardiovascular disease, and stroke. The average BMI was overweight (24.22 ± 4.09 kg/m^2).

From laboratory findings, the median of calcium, phosphate, and albumin level was 9.80 mg/dL, 5.50 mg/dL, and 3.9 mg/dL, respectively. Using ELISA, the median of Fetuin-A and IL-6 concentration was 235.0 pg/mL (range 78–756) and 70.7 pg/mL (range 25.4–898.0), respectively, with the average of 259.99 ± 119.36 pg/mL and 99.64 ± 115.51 pg/mL, respectively.

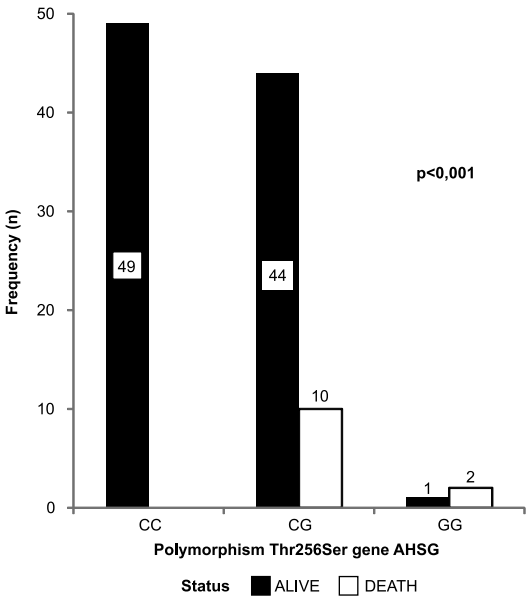

Figure 1. Frequency of distribution of Fetuin-A Thr256Ser gene polymorphism based on life status

The restriction enzyme analysis (SacI) on the Fetuin-A gene showed the C Allele did not contain the SacI enzyme. Thus it was not cut off as 405 bp fragment, while G allele containing SacI enzyme was cut off as 193 and 212 bp fragment. Through PCR-RFLP, distribution frequency of 3 Fetuin-A gene genotypes was obtained 46.2% for Thr/Thr (C homozygous allele/genotype CC), 50.9% for Thr/Ser (G heterozygous allele/CG genotype) and 2.8% for Ser/Ser (G homozygous allele/ GG genotype).

Survival on chronic haemodialysis patients

During nine months of follow up, 12 (11.3%) patients did not survive, while 94 (88.7%) survived until the end of the research. The mean survival of patients since the begging of the study was 8.49±1.53 months (a median of 9 months; range 1-9 months).

Impact of various factors on nine months survival rate

A distinction of proportion between the patients who were still alive with those who did not survive was found. Among 12 deceased patients, most of them carried GG genotype with 8.87 times mortality risk compared to those with CC+CG genotypes (p=0.005). IL-6 level significantly affected the proportion of mortality in patients. The group of patients with IL-6 level ≥86.9 pg/mL had a higher mortality rate with 3.64 times mortality risk compared to those with IL-6 level of <89.6 pg/mL (p=0.03). Even if the difference of proportion was statistically insignificant (p≥0.05), among 12 deceased patients, more of them had Fetuin-A level <235 pg/mL, with 3.36 times mortality risk compared to those with Fetuin-A level ≥235 pg/mL (p=0.07). Besides, there were more of them with albumin level <3.9 mg/dL, with 3.04 times mortality risk compared to those with albumin level albumin ≥3.9mg/dL (Table 1).

All patients (100%) with CC genotype were still alive until this research ended, while many patients with GG genotype were dead (Figure 2).

The Kaplan-Meier curve of 9 months survival rate for maintenance haemodialysis patients ba-

Table 1. Comparison of maintenance dialysis patients based on life status and univariate cox regression for mortality risk factor

Variable	Life status		HR (95% CI)	p
	Survive (n=94)	Dead (n=12)		
Polymorphism, n (%)			8.87 (1.94-40.58)	0.005
CC+ CG Genotype	93 (90.3)	10 (9.7)		
GG Genotype	1 (33.3)	2 (66.7)		
Vascular calcification	6.1 ± 5.7 6 (0-22)	10.25 ± 6.3 11 (0-18)	2.28 (0.50-10.40)	0.29
No calcification	30 (93.8)	2 (6.2)		
Calcification	64 (86.5)	10 (13.5)		
Fetuin-A (pg/mL)	265.9 ± 124.5 238.5 (78 – 756)	213.6 ± 47.5 217.5 (113 – 300)	3.36 (0.91-12.40)	0.07
≥235	74 (91.4)	7 (8.6)		
<235	20 (80.0)	5 (20.0)		
IL-6 (pg/mL)	97.7 ± 118.6 69.5 (25.4 – 898)	115.1 ± 90.3 91.2 (48.2 – 386)	3,64 (1.16-11.48)	0.03
<86.9	69 (93.2)	5 (6.8)		
≥86.9	25 (78.1)	7 (21.9)		
Albumin (mg/dL)	3.9 ± 0.3 3.9 (3.2 – 4.9)	3.8 ± 0.5 3.7 (2.9 – 4.8)	3.04 (0.91-10.09)	0.07
≥ 3.9	58 (93.5)	4 (6.5)		
< 3.9	36 (81.8)	8 (18.2)		
Calcium (mg/dL)	9.7 ± 0.7 9,8 (8.0 – 10.9)	9.8 ± 0.8 9.8 (8.2 – 10.8)	0.94 (0.30-2.96)	0.92
≤9.5	38 (88.4)	5 (11.6)		
>9.5	56 (88.9)	7 (11.1)		
Phosphate (mg/dL)	5.4 ± 0.6 5.4 (4.0 – 6.8)	5.5 ± 0.7 5.7 (4.2 – 6.8)	1.68 (0.53-5.28)	0.38
≤5.5	52 (91.2)	5 (8.8)		
>5.5	42 (85.7)	7 (14.3)		
Age (year)	53.5 ± 11.8	56.6 ± 8.3	1.36 (0.43-4.28)	0.60
<60	61 (89.7)	7 (10.3)		
≥60	33 (86.8)	5 (13.2)		
Duration of dialysis (months)	68.1 ± 34.8 54.5 (34 - 237)	79.9 ± 32.5 78.5 (40 - 146)	1.62 (0.51-5.09)	0.41
<60	52 (91.2)	5 (8.8)		
≥60	42 (85.7)	7 (14.3)		
Diabetes mellitus			0.03	0.25
No	71 (85.5)	12 (14.5)	(0.00-10.69)	
Yes	23 (100.0)	0 (0.0)		
BMI (kg/m2)	24.3 ± 4.2 23.8(16.7 – 42.7)	23.6 ± 3.6 22.1 (18.4 – 30.3)	1.28 (0.38-4.24)	0.69
Underweight-normal	27 (87.1)	4 (12.9)		
Overweight-obese	67 (89.3)	8 (10.7)		

HR, Hazard Ratio; 95%CI, Confidence Interval; BMI, body mass index

sed on Fetuin-A genotype in 106 patients showed that patients with CC genotype had the highest cumulative survival rate at the end of the research, while patients with GG genotype had the worst survival rate compared to the other (p<0.001).

Table 2. Multivariate Cox Regression time independent analysis result

Model	Variable	HR	95% CI	p
	IL6 level(≥86,9 pg/mL)	4.15	0.83-20.77	0.08
	Fetuin-A level(<235 pg/mL)	0.50	0.09-2.85	0.43
1	Albumin level (< 3.9 mg/dL)	2.18	0.61-7.79	0.23
	Fetuin-A gene polymorphism (GG genotype)	4.12	0.65-26.26	0.13
	IL6 level (≥86,9 pg/mL)	2.62	0.75-9.11	0.13
2	Fetuin-A gene polymorphism (GG genotype)	3.25	0.59-12.81	0.18
	Albumin level(< 3.9 mg/dL)	2.21	0.61-7.89	0.22
	IL6 level (≥86,9 pg/mL)	2.83	0.82-9.77	0.10
3	Fetuin-A gene polymorphism (GG genotype)	4.64	0.90-23.95	0.07
4	Fetuin-A gene polymorphism (GG genotype)	8.87	1.94-40.58	0.005

HR, Hazard Ratio; 95% CI, Confidence Interval;

Various factors contribute in 9 months survival rate

To find out the risk factors that contribute to 9-month survival rate, multivariate analysis with Cox Regression Time Independent using a backward wald method was performed, including variable with p ≤0.25 on previous bivariate analysis: Fetuin-A gene polymorphism, Fetuin-A level, IL-6 level, and albumin level. The analysis revealed that Fetuin-A gene polymorphism (GG genotype) showed a dominant impact on the mortality of maintenance haemodialysis patients with Hazard Ratio, HR=8.87, and p=0.005. The magnitude of Fetuin-A gene polymorphism impact on mortality of maintenance haemodialysis patients motivated us to analyze patient characteristics based on Fetuin-A gene polymorphism. The patients with CC genotype were more likely to have diabetes mellitus, 19 (82.6%), with the

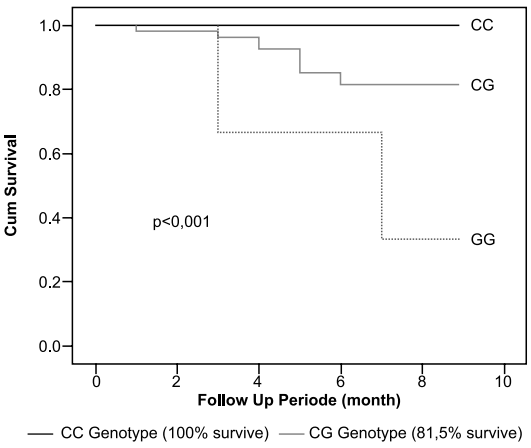

Figure 2. Kaplan-Meier Curve of 9-month survival rate in maintenance dialysis patients based on Fetuin-A genotype

highest level of Fetuin-A (mean 319.1 ± 147.8 pg/mL and median 266.0 pg/mL), with the lowest mean of IL-6 level (61.2 ± 34.3 pg/mL), and also the highest mean (4.0 ± 0.3 mg/dL) and median (4.0 mg/dL) of albumin level compared to other genotypes. On the contrary, the group of patients with GG genotype were less likely to have diabetes mellitus (p<0.001), had the lowest mean and median of Fetuin-A level (p<0.001), the highest mean of IL-6 (p<0,001), and also the lowest mean and median of albumin (p=0,006) compared to other Fetuin-A genotype (Table 3).

Table 3. Maintenance dialysis patients characteristics based on Fetuin-A gene polymorphism

Variable	Fetuin-A Thr256Ser gene polymorphism			p
	CC (n= 49)	CG (n=54)	GG (n=3)	
Age (Years), mean±SD, median (range)	55.4 ± 11.0 51.0 (8 – 71)	52.7 ± 11.7 57.5 (21 – 75)	50.0 ± 14.0 44 (40 – 66)	0.50
Diabetes mellitus				<0.001
No	30 (36.4)	50 (60.2)	3 (3.6)	
Yes	19 (82.6)	4 (17.5)	0 (0.0)	
BMI (kg/m2), mean±SD, median (range)	24.7 ± 4.0 24.0 (18–41.4)	23.7 ± 4.1 23.1 (16.7–42.7)	29.0 (18.4–29.3)	0.20
BMI				0.17
Underweight-normal	33 (44.0)	41 (54.7)	1 (1.3)	
Overweight-obese	16 (51.6)	13 (41.9)	2 (6.5)	
Fetuin-A (pg/mL), mean±SD, median (range)	319.1 ± 147.8 266.0 (184–756)	216.5 (111 – 328)	120.3 ± 4.5 113.0 (78 – 170)	<0.001
IL-6 (pg/mL), mean±SD, median (range)	61.2 ± 34.3 61.0 (38 – 237)	68.8 ± 35.7 53.0 (34 – 224)	74.3 ± 26.7 62 (56 – 105)	<0.001
Albumin (mg/dL), mean±SD, median (range)	4.0 ± 0.3 4.0 (3.3 – 4.9)	3.9 ± 0.3 3.9 (3.2 – 4.8)	3.4 ± 0.5 3.5 (2.9 – 3.8)	0.006

DISCUSSION

The results of this study have shown a significant independently dominant impact between Fetuin-A Thr256Ser gene polymorphism and the survival rate of maintenance haemodialysis patients. We found mortality rate for nine-month follows up was around 11.3%. This rate is lower compared to the survey by Sibarani et al. in 2018, with a 36.6% one-year mortality rate (10). Unfortunately, this mortality rate is higher than in developed countries. In Japan, one-year mortality rate in 2009 is reported only at 9.6% (11). Although haemodialysis technique and the facility have been developed in Indonesia, the mortality rate remains high among chronic haemodialysis patients. Several circumstances are thought to cause a high mortality rate in chronic haemodialysis patients in Indonesia compared to those in a developed country, such as the difference in demographic characteristics, referral pattern, and haemodialysis pattern in practice (12,13).

During nine months of follow-up, the survival rate from this research was 88.7%. The mean survival of chronic haemodialysis patients is about 5-10 years (10). In European countries over the past decade, the five-years survival of haemodialysis patients has been improved (10). Two years of survival of haemodialysis patients in Europe increased from 80.6% to 82.2% between 2006-2010 (14). According to the United States Renal Data System 2015, five years of survival rose from 35% to 40% among haemodialysis patients and 75% to 87% among donor transplant patients (15).

Generally, the high risk of mortality and morbidity of cardiovascular disease (CVD) in CKD correlate with a high prevalence of classical risk factors (16). Some factors related to uremia also play critical roles such as hypoalbuminemia, inflammation, and vascular calcification (VC) that correlate with a low level of Fetuin-A.(17). Weng et al. have shown that a group of patients in chronic haemodialysis with albumin serum level <4.0 g/dL had a higher mortality rate caused by infection and CVD (18). Amaral et al. have also shown that a decrease in mortality and hospitalization risk occurred in patients with albumin serum level ≥3.5 g/dL and the lowest mortality risk when albumin serum level ≥4.0 g/dL (19). Even more, De Mutsert et al. study on 700 patients in 38 haemodialysis centres in the Netherlands revealed that the decrease of 1 g/dL albumin serum correlates with a 47% increase in mortality risk (20). Albumin plasma concentration below 4 g/dL correlates with a higher mortality rate, whereas the rate was higher if the concentration was below 3 g/dL (21). In line with the previous study, the present study shows that patients with albumin level <3.9 mg/dL have a 3.04 times mortality risk compared to those with albumin level ≥3.9 mg/dL, though this implication is dependent since multivariate analysis showed no correlation.

Systemic inflammation is commonly found in CKD patients with maintenance haemodialysis (22). A study by Beberashvili et al. has shown chronic inflammation marked with an increased level of IL-6 correlate with all-cause mortality in

stable chronic haemodialysis patients (23). Research by Honda et al. observed albumin serum, CRP, IL-6, and Fetuin-A showing the same result. They proved that an increased level of IL-6 was a better mortality predictor compared to hs-CRP (24). The same effect is seen in this study, with the mortality rate of patients with IL-6 level <86.9 pg/mL higher compared to those whose IL-6 level ≥86.9 pg/mL.

Inflammation is also the leading cause of the low level of serum Fetuin-A in CKD patients on maintenance haemodialysis. A study by Stenvinkel et al. proved that there was a significant negative correlation between the Fetuin-A level and IL-6 level (7). In line with this finding, Metry et al. found that a low level of Fetuin-A correlated with mortality in chronic haemodialysis patients (25). In a study by Marechal et al. the same conclusion is seen where a low level of Fetuin-A correlated with aortic calcification and could predict mortality risk of a patient who had undergone renal replacement therapy (26).

Fetuin-A is also called a negative acute phase reactant due to its concentration in circulation decreasing in acute and chronic inflammation, as seen in CKD patients with maintenance haemodialysis (7). Previous studies and this study proved that patients with chronic haemodialysis had a lower level of Fetuin-A compared to healthy control (27). As IL-6 increases and the level of Fetuin-A decreases, the patient survival rate will decline. Ketteler et al. also proved that a low level of Fetuin-A could predict an increase in mortality due to CVD in haemodialysis patients. Their study suggests that deficiency of Fetuin-A contributes to declining of vascular wall elasticity, which is an early sign of uremic vasculopathy and CVD risk factor in CKD patients on maintenance haemodialysis (28,29).

According to Honda et al. the cut-off value of Fetuin-A 0.19 g/L could predict haemodialysis patient's mortality with sensitivity/specificity around 64.3%/68.7%. The lower Fetuin-A level, the sooner death will occur, and an increase of 0.1 g/L will decrease all-cause mortality around 13% (1). However, Blaha et al. study revealed a small difference; they proved that a low level of circulating Fetuin-A was a significant predictor of early mortality, but not for late mortality in patients with chronic haemodialysis. A significantly lower level of Fetuin-A was found in the deceased patients compared to a patient who survived until the 12th month. However, for the patient who did not survive until the 24th month, the Fetuin-A level showed no significant difference between the deceased patient and alive patient (1).

In an attempt to prove the mechanism of increased mortality risk in chronic haemodialysis patients, restriction enzyme analysis is used to detect Fetuin-A Thr256Ser gene polymorphism. Genetic alteration is thought to be the essential cause of the low level of circulating Fetuin-A. In Maharem et al. study, a statistically significant correlation between Fetuin-A Thr256Ser gene polymorphism and Fetuin-A serum level where patients with G allele had lower Fetuin-A level compared to those with CC genotype was found (17). It is also reported that chronic haemodialysis patients carrying allele 256ser have a lower level of Fetuin-A compared to those who carry 256thr allele. This high level of inflammation in these patients causes higher mortality risk compared to patients in chronic haemodialysis with a 256Thr allele with signs of inflammation (25).

This study proved that Fetuin-A Thr256Ser gene polymorphism showed a statistically significant association with survival of chronic haemodialysis patients. Even more, multivariate analysis revealed that Fetuin-A Thr256Ser gene polymorphism (GG genotype) was an independent dominant factor influencing chronic haemodialysis patient's mortality with the HR of 8.87 times. The mechanism of high mortality caused by the GG genotype remains unclear whether it is caused by race so that the GG genotype becomes lethal in Indonesia or because of selection bias in choosing the study sample. Our patient with GG genotype had a low Fetuin-A level and albumin level median, as well as a high mean of IL-6 (Table 3). Maybe these conditions cause increased mortality in our patients. Stenvinkel et al. first reported that Thr256Ser gene polymorphism, such as Ser/Ser (G allele) in the Fetuin-A gene, increased the risk of CVD mortality associated with a low level of Fetuin-A in Swedish chronic haemodialysis patients (7). The mechanism of low Fetuin-A level caused by the GG genotype in the Fetuin-A gene remains unclear. Because the SNP rs4918 position lies in exon, it does not influence the production of Fetuin-A directly. In another case,

SNP rs2248690 lies in the promoter, where it directly modified the Fetuin-A transcription by changing AP-1 affinity. However, another study suggests that SNP rs2248690 and SNP rs4918 have linkage disequilibrium, which had the potential to interfere with DNA binding in some transcription factors (TFs), including AP-1 and Erα. A study has proved that the transcriptional complex consists of ERα and AP-1contributes to Fetuin-A transcription (3).

This study revealed that there was a significant independently dominant impact between Fetuin-A Thr256Ser gene polymorphism and the survival rate of chronic dialysis patients. The results suggest that genotype variation of Fetuin-A gene could be a potential marker to identify high mortality risk in Indonesia's maintenance dialysis patients, especially in Medan. With early control of factors related to Fetuin-A genotype variation, the survival rate of CKD patients undergoing dialysis should improve.

FUNDING

No specific funding was received for this study.

TRANSPARENCY DECLARATION

Conflict of interest: None to declare.

REFERENCES

1. Bláha V, Mistrík E, Dusilová-Sulková S, Kalousová M, Andrýs C, Bláha M, Sobotka L. Circulating fetuin-A predicts early mortality in chronic haemodialysis patients. Clin Biochem 2009; 42:996-1000.
2. Osawa M, Yuasa I, Kitano T, Henke J. Haplotype analysis of the human α2-HS glycoprotein (fetuin) gene. Ann Hum Genet 2001; 65:27-34.
3. Jensen MK, Jensen RA, Mukamal KJ,Guo X, Yao J, Sun Q, Cornelis M, Liu Y, Chen MH, Kizer JR, Djoussé L, Siscovick DS, Psaty BM, Zmuda JM, Rotter JI, Garcia M, Harris T, Chen I, Goodarzi MO, Nalls MA, Keller M, Arnold AM, Newman AB, Hoogeveen RC, Rexrode KM, Rimm EB, Hu FB, Ramachandran VS, Katz R, Pankow JS, Ix JH. 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; 26:2156-63.
4. Schinke T, Amendt C, Trindl A, Pöschke O, Müller-Esterl W, Jahnen-Dechent WJJoBC. The serum protein α2-HS glycoprotein/fetuin inhibits apatite formation in vitro and mineralizing calvaria cells, a possible role in mineralization and calcium homeostasis. J Biol Chem 1996; 271:20789-96.
5. Ix JH, Shlipak MG, Brandenburg VM, Ali S, Ketteler M, Whooley MAJC. Association between human fetuin-A and the metabolic syndrome data from the heart and soul study. Circulation 2006; 113:1760-7.
6. Mehrotra RJKi. An emerging role for fetuin-A as a contributor to morbidity and mortality in chronic kidney disease. Kidney Int 2007; 72:137-40.
7. Stenvinkel P, Wang K, Qureshi AR, Axelsson J, Pecoits-Filho R, Gao P, Barany P, Lindholm B, Jogestrand T, Heimbürger O, Holmes C, Schalling M, Nordfors L. Low fetuin-A levels are associated with cardiovascular death: impact of variations in the gene encoding fetuin. Kidney Int 2005; 67:2383-92.
8. Verduijn M, Prein RA, Stenvinkel P, Carrero JJ, le Cessie S, Witasp A, Nordfors L, Krediet RT, Boeschoten EW, Dekker FW. Is fetuin-A a mortality risk factor in dialysis patients or a mere risk marker? A Mendelian randomization approach. Nephrol Dial Transplant 2011; 26:239-45.
9. US Renal Data System. USRDS 2010 Annual data report: atlas of chronic kidney disease and end-stage renal disease in the United States. 2010. https://www.usrds.org/2010/view/ (08 August 2018)
10. Sibarani H, Muzasti R, Lubis H. Association between neutrophil-to-lymphocyte ratio and survival rate patient haemodialysis in Adam Malik general hospital in 2016. In: Proceedings of IOP Conference Series: Earth and Environmental Science, Medan, Indonesia, March 2018.
11. Robinson B, Zhang J, Morgenstern H, Bradbury BD, Ng LJ, McCullough KP, Gillespie BW, Hakim R, Rayner H, Fort J, Akizawa T, Tentori F, Pisoni R.World-wide, mortality is a high risk soon after initiation of haemodialysis. Kidney Int 2014; 85:158-65.
12. Indonesian Renal Registry. Report on Indonesian Renal Registry. 2017 https://www.indonesianrenalregistry.org/ (08 August 2018)
13. Prodjosudjadi W, Suhardjono AJE, Disease. End-stage renal disease in Indonesia: treatment development. Ethn Dis 2009; 19(1 Suppl 1):S1-33-6
14. Pippias M, Jager KJ, Kramer A, Leivestad T, Sánchez MB, Caskey FJ, Collart F, Couchoud C, Dekker FW, Finne P, Fouque D, Heaf JG[1], Hemmelder MH, Kramar R, De Meester J, Noordzij M, Palsson R, Pascual J, Zurriaga O, Wanner C, Stel VS. The changing trends and outcomes in renal replacement therapy: data from the ERA-EDTA Registry. Nephrol Dial Transplant 2016; 31:831-41.
15. US Renal Data System, USRDS 2015 Annual data report: atlas of chronic kidney disease and end-stage renal disease in the United States. 2015 https://www.usrds.org/2015/view/ (08 August 2018)
16. Georgianos PI, Agarwal R. Hypertension in chronic kidney disease: Nephrology Dialysis Transplantation notable 2017 advances. Nephrol Dial Transplant 2019; 34:1-4.
17. Maharem DA, Gomaa SH, El Ghandor MK, Mohamed EI, Matrawy KA, Zaytoun SS, Nomeir HM. Association of serum fetuin-A and fetuin-A gene polymorphism in relation to mineral and bone disorders in patients with chronic kidney disease. The EJMHG 2013; 14:337-52.

18. Weng CH, Hu CC, Yen TH, Hsu CW, Huang WH. Nutritional predictors of mortality in long term haemodialysis patients. Sci Rep 2016;6:35639.
19. Amaral S, Hwang W, Fivush B, Neu A, Frankenfield D, Furth S. Serum albumin level and risk for mortality and hospitalization in adolescents on haemodialysis. Clin J Am Soc Nephrol 2008; 3:759-67.
20. de Mutsert R, Grootendorst DC, Indemans F, Boeschoten EW, Krediet RT, Dekker FW; Netherlands Cooperative Study on the Adequacy of Dialysis-II Study Group. Association between serum albumin and mortality in dialysis patients is partly explained by inflammation, and not by malnutrition. J Ren Nutr 2009; 19:127-35.
21. Chung SH, Chu WS, Lee HA, Kim YH, Lee IS, Lindholm B, Lee HB. Peritoneal transport characteristics, comorbid diseases, and survival in CAPD patients. Perit Dial Int 2000; 20:541-7.
22. Lau WL, Kalantar-Zadeh K, Vaziri NDJN. The gut as a source of inflammation in chronic kidney disease. Nephron 2015; 130:92-8.
23. Beberashvili I, Sinuani I, Azar A, Yasur H, Shapiro G, Feldman L, Averbukh Z, Weissgarten J. IL-6 levels, nutritional status, and mortality in prevalent haemodialysis patients. Clin J Am Soc Nephrol 2011; 6:2253-63.
24. Honda H, Qureshi AR, Heimbürger O,_Barany P, Wang K, Pecoits-Filho R, Stenvinkel P, Lindholm B. Serum albumin, C-reactive protein, interleukin 6, and fetuin-A as predictors of malnutrition, cardiovascular disease, and mortality in patients with ESRD. Am J Kidney Dis 2006; 47:139-48.
25. Metry G, Stenvinkel P, Qureshi A, Carrero JJ, Yilmaz MI, Bárány P, Snaedal S, Heimbürger O, Lindholm B, Suliman ME. Low serum fetuin-A concentration predicts poor outcomes only in the presence of inflammation in prevalent haemodialysis patients. Eur J Clin Invest 2008; 38:804-11.
26. Maréchal C, Schlieper G, Nguyen P, Krüger T, Cochе E, Robert A, Floege J, Goffin E, Jadoul M, Devuyst O. Serum fetuin-A levels are associated with vascular calcifications and predict cardiovascular events in renal transplant recipients. Clin J Am Soc Nephrol 2011; 6:974-85.
27. Hermans M, Brandenburg V, Ketteler M, Kooman JP, van der Sande FM, Boeschoten EW, Leunissen KM, Krediet RT, Dekker FW; Netherlands cooperative study on the adequacy of Dialysis (NECOSAD). Association of serum fetuin-A levels with mortality in dialysis patients. Kidney Int 2007; 72:202-207.
28. Ketteler M, Bongartz P, Westenfeld R, Wildberger JE, Mahnken AH, Böhm R,–Metzger T, Wanner C, Jahnen-Dechent W, Floege J. Association of low fetuin-A (AHSG) concentrations in serum with cardiovascular mortality in patients on dialysis: a cross-sectional study. Lancet 2003; 361:827-33.
29. Blacher J, Guerin AP, Pannier B, Marchais SJ, London GMJH. Arterial calcifications, arterial stiffness, and cardiovascular risk in end-stage renal disease. Hypertension 2001; 38:938-42.