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Relationship between High Sensitivity C-Reactive Protein and Total Testosterone Levels in Male Patients with Stage V Chronic Kidney Disease

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ABSTRACT

The incidence of decreased total testosterone level increases in stage V Chronic Kidney Disease (CKD) patients. Decreased total testosterone levels is influenced by uremia and hemodialysis bio incompatibility through an increase in the inflammatory mediator hs-CRP. Obesity and age are risk factors of CKD incidence, which can directly affect testosterone level. This study aimed to analyze the relationship between hs-CRP, serum urea, age, obesity, and hemodialysis duration with decreased total testosterone levels in stage V CKD patients. This observational study with cross-sectional approach was performed are 60 stage V CKD patients treated in Dr. Moewardi General Hospital, Surakarta on November 2020. The data were analyzed with 2x2 table test followed by multivariate analysis using logistic regression. The total testosterone and hsRP levels in this study were measured using ECLIA and immunoturbidimetric assay, respectively. This study found decreased total testosterone levels in 21 (37%) stage V CKD patients with total testosterone levels < 3 ng/mL. This study found that hs-CRP level [Prevalence Ratio/PR 3.656 (95% Confidence Interval/CI: 1.202-11,124; p=0.020)]; obesity [PR 4.156 (95% CI: 1.272-13.581; p=0.015)] and urea [PR 4.474 (95% CI: 1.273-15.728; p=0.015)] were significantly associated with decreased total testosterone level of < 3 ng/mL in stage V CKD patients. However, there was no significant relationship between age and duration of hemodialysis with p=0.694, PR <1, and CI 95% < 1 (p=0.018). Therefore, it was concluded that in patients with stage V CKD, hs-CRP levels ≥ 0.65 mg/dL, serum urea ≥ 120 mg/dL, and obesity correlate with decreased total testosterone levels while age was not associated with decreased total testosterone level. The hemodialysis duration was not a risk factor for decreased total testosterone. The results of the multivariate logistic regression analysis of adjustments to the urea variable 120 mg/dL showed a PR of 5.734 (95% CI: 1.31-25.114; p=0.020), obesity variable with a PR of 6.649 (95% CI: 1.593-27.75; p=0.009) and the hs-CRP variable 0.65 mg/dL with a PR of 4.324 (95% CI: 1.189-15,735).

Keywords: Chronic kidney disease, hs-CRP, total testosterone, urea, obesity, hemodialysis

INTRODUCTION

Chronic Kidney Disease (CKD) is a condition characterized by a decrease in the Glomerular Filtration Rate (GFR), with a value < 60 mL/min/1.73 m^2 and a marker of kidney damage that occurs for more than three months.[1] Results of the meta-analysis by Hill *et al.* showed that the global prevalence of CKD incidence was 13.4% with the prevalence of stage 3 to stage 5 CKD patients was 10.6%.[2]

End-stage chronic kidney disease or End-State Renal Disease (ESRD) is a state of severely decreased kidney function with a GFR < 15 mL/min/1.73 m^2 resulting in uremia and the need for renal replacement therapy (dialysis) to repair kidney function in eliminating body toxin.[3] Hemodialysis in the long term has a greater risk of causing an inflammatory reaction originating from the fistula, dialysis membrane, and infection. This reaction is associated with increased levels of inflammatory markers such as serum CRP.[4]

Progressive renal impairment is associated with worsening systemic inflammation and elevated levels of C-Reactive Protein (CRP) detected along with other acute-phase proteins. Monitoring CRP levels in CKD patients, especially in the late stage, has been implemented because CRP is an inflammatory marker which has a stable condition and does not show significant circadian variability.[5,6]

Previous studies have shown that approximately 44% of male patients with end-stage CKD exhibit hypogonadism/testosterone deficiency. Hypogonadism is characterized by low total serum testosterone with levels < 300 ng/dL or 10.4 nmol/L followed by one clinical signs or symptom. Symptoms of hypogonadism that appear after puberty are: Sexual dysfunction such as libido, erectile dysfunction, difficulty of orgasm and ejaculation; A reduction in energy, vitality or stamina; Depressed mood; and Concentration difficulty.[7,8]

The decrease of testosterone levels in patients with CKD itself has multifactorial causes, such as

decreased clearance of prolactin in patients with CKD, which can inhibit the production of luteinizing Hormone (LH) thereby reducing testosterone production; uremia itself is able to directly inhibit LH receptors in Leydig cells, resulting in an imbalance in testosterone production, in addition to its ability to cause progressive systemic inflammation. Proinflammatory cytokines also have a role in the occurrence of testosterone deficiency by reducing the secretion of Gonadotropin-Releasing Hormone (GnRH).[7,9]

The gradual decrease in circulating testosterone levels and sperm production is not caused by decreased stimulation, but possibly due to degenerative processes associated with aging. Older men are more likely to have low testosterone levels.[10] Obesity is another risk factor, which also affects testosterone levels. Adipose tissue affects testosterone levels by increasing the aromatization of testosterone to estradiol, because the aromatase enzyme is concentrated in adipocytes and this reduces serum and tissue testosterone levels.[11]

Testosterone deficiency is associated with the risk of Cardiovascular Disease (CVD) in CKD. The mortality rate for stage V CKD is above 20% per year and more than half of deaths are CVD-related.[12] Based on the background above, this study aimed to determine the relationship between serum hs-CRP and risk factors (serum urea, age, obesity and duration of hemodialysis) with the incidence of decreased total testosterone levels in patients with end-stage CKD/stage V.

METHODS

This research was performed in November 2020 at the Clinical Pathology Laboratory Installation, Dr. Moewardi Regional General Hospital, Surakarta. This study was an observational analytic study with a cross-sectional approach. Research subjects in this study were selected by consecutive sampling and based on inclusion and exclusion criteria. The inclusion criteria of the research subjects were patients with end-stage CKD who had received hemodialysis therapy, male, aged more than 20 years and agreed to participate in the study by signing informed consent. Exclusion criteria for research subjects were: patients with a history of or current evidence of diabetes mellitus, autoimmune disease, Human Immunodeficiency Virus (HIV), cancer, liver disorders, alcohol consumption, chemotherapy drugs, testosterone and estrogen therapy. Patients with acute infectious inflammation were also excluded from this study because hs-CRP is an acute phase reaction that can lead to biased results.

A total of 3 mL of venous blood was taken in the morning without prior fasting. Blood was collected in a tube without anticoagulant. hs-CRP levels were measured using ADVIA 1800 with an immunoturbidimetric method and total testosterone levels were measured using COBAS e 411 analyzer with Electro Chemiluminescence Immunoassay (ECLIA) method. Laboratory tests were preceded by precision tests and analytical accuracy tests. The data on the characteristics of the research subjects with normal distribution were presented as mean and standard deviation, but data with abnormal distribution were presented as the median (minimum-maximum). Independent T-test or Mann-Whitney test was used to analyze the differences in the variables of age, obesity, hs-CRP, urea, and duration of hemodialysis in the group with decreased total testosterone levels and normal total testosterone levels. Cut-off values for hs-CRP and serum urea levels were determined using the Receiver Operating Curve (ROC), the widest Area Under the Curve (AUC) with the maximum cut-off point. Chi-Square test was used to analyze the relationship between age, obesity, hs-CRP, urea and duration of hemodialysis with decreased total testosterone levels and continued with multivariate logistic regression analysis of variables with $p < 0.25$. Statistical analysis was carried out using a computer program, $p<0.05$, and CI, which did not include number one was stated as significant. This study has received ethical approval from the Health Research Ethics Commission of Dr. Moewardi Hospital with number 1.195/X/HREC/2020.

RESULTS AND DISCUSSIONS

The basic characteristics and variables of the research subjects were described in Table 1. Subjects in this study consisted of 60 male patients with a mean age of 50.47±15.7. Most of the patients with end-stage CKD in this study had hypertension. The mean hemoglobin level in this study was 8.62±1.38 g/dL, indicating the presence of anemia. Anemia is a common complication of late-stage CKD, with symptoms such as fatigue and shortness of breath. The pathogenesis of anemia in CKD is complex and influenced by several things, most notably a decrease in erythropoietin levels produced by the kidneys, which is exacerbated by the presence of hepcidin-related metabolic disorders in the chronic inflammation.[13]

The mean creatinine levels ranged at 11.32±3.93 mg/dL and the mean eGFR levels were 7.10±2.59 mL/min/1.73 m^2. GFR levels are the best marker of renal function and their decreased levels are

associated with increased mortality in CKD patients. A decrease in GFR <15 mL/min is an indication of the need for renal replacement therapy.[14]

The average body mass index in this study was 22.73±3.27 kg/m^2 and the mean total testosterone level was 3.82±2.09 ng/mL. Obesity was found in 14 (23%) subjects but not found in 46 (77%) subjects. The median of hs-CRP levels in this study was 0.37 (0.03-7.57) mg/dL, the mean urea level was 140.40±67.47 and the median duration of hemodialysis was 13 (1-54) months.

The data on the characteristics of the research variables presented in Table 2 showed significant differences in the variables of obesity, hs-CRP, urea and hemodialysis with p=0.015; 0.007; 0.001 and 0.038 respectively, but no significant difference for the age (p=0.788). A study by Odujoko *et al*. showed increased CRP levels in CKD patients with a mean of 0.54±2.17 mg/dL.[15] In addition, a study by Carrero *et al*. obtained a similar result, showing a significant difference in the CRP levels in patients with testosterone deficiency and non-testosterone deficiency with p<0.0001.[7]

The ROC curve showed that the cut-off value for serum urea levels was 120 mg/dL with an AUC of 0.700 (95% CI; 0.544-0.826; p=0.011) with a sensitivity of 67% and a specificity of 51% (Figure 1). In addition, the cut-off value for serum hs-CRP levels was 0.65 mg/dL with an AUC of 0.714 (95% CI; 0.571-0.857; p=0.007) with a sensitivity of 62% and specificity of 69% (Figure 2). These two cut-off values were then used for the calculation of the bivariate test on the research variables.

The bivariate analysis in Table 3 shows a statistically significant relationship between obesity, hs-CRP 0.65 mg/dL and urea 120 mg/dL with p=0.015; 0.020 and 0.015, respectively. However, hemodialysis with a PR of 0.135 (95% CI: 0.024-0.746; p=0.018) showed PR < 1 and the confidence interval range, which did not include the number one, indicating that the factor studied was a protective factor, not a risk factor. In addition, there was no statistical significance of age in this study (p=0.694).

Bivariate analysis between age and total testosterone levels revealed a PR of 0.800 (95% CI: 0.263-2.435; p=0.694) indicating that there was no significant relationship between age and total testosterone levels in stage V CKD patients. A study by Mehta *et al*. found no significant difference between the 2 groups (group 1 aged 18-29 years and group 2 aged 45-65 years) with a decrease in total testosterone levels (p=0.11) among the healthy male population; this was because the total testosterone levels in this study were not measured at the same time in the morning, the gold standard Liquid

Table 1. Basic characteristics of research subjects

Variable[a]	Mean±SD[a]
Hemoglobin (g/dL)	8.62±1.38
Creatinine (mg/dL)	11.49±3.72
eGFR (mL/menit)	7.10±2.59
BMI (kg/m^2)	22.73±3.27
Total testosterone (ng/mL)	3.82±2.09

Note: g: gram; dL: deciliter; eGFR: estimated Glomerolus Filtration Rate; mL: milliliter, ng: nanogram, mg: milligram, BMI: Body Mass Index, kg: kilogram, m: meter, a: normal distribution (mean±SD)

Table 2. Characteristics of research variables

Parameter		Total Testosterone		P
		<3 ng/mL	≥ 3 ng/mL	
Total	60 (100%)	21 (37%)	39 (63%)	
Age (years)[a]	50.47±15.7	49.9±15.4	50.7±16.09	0.788
Obesity[c]				0.015*
Yes	14 (23%)	10 (58.8%)	14 (23%)	
No	46 (77%)	11 (25.6%)	46 (77%)	
hs-CRP (mg/dL)[b]	0.37 (0.03-7.57)	1.32 (0.08-7.54)	0.25 (0.03-7.57)	0.007*
Urea (mg/dL)[a]	140,40±67.47	183,9±92.3	116,9±31.2	0.001*
Hemodialysis[b] (months)	13 (1-54)	7 (1-50)	15 (1-54)	0.038*

Note: ng: nanogram; mL: milliliter; mg: milligram; dL: desiliter; hs-CRP: high sensitivity C-Reactive Protein
a: normal distribution, difference test with independent T-test
b: abnormal distribution, difference test with Mann-Whitney
c : categorical data reported in percentage (%)
*p<0.05 was significant

Chromatography-Mass Spectrometry (LC-MS) was not used in the measurement of total testosterone levels, and history of previous total testosterone levels was unknown.[16]

Results of bivariate analysis in this study showed that there was a significant relationship between obesity and total testosterone levels (p=0.015). Similar results were also shown by the research of Dwipayana *et al.* in Korea, which found an inverse correlation between total testosterone levels and an increase in body size/abdominal obesity (r=-0.093, p<0.001) in 6967 research subjects.[17] Obesity is the most important factor causing testosterone deficiency. Testosterone deficiency in obese patients is associated with visceral fat dysfunction, insulin resistance, leptin resistance, and the presence of proinflammatory cytokines. Testosterone deficiency can lead to increased adipogenesis and visceral obesity. The most consistent effect of androgens on body fat accumulation is the activation of Androgen

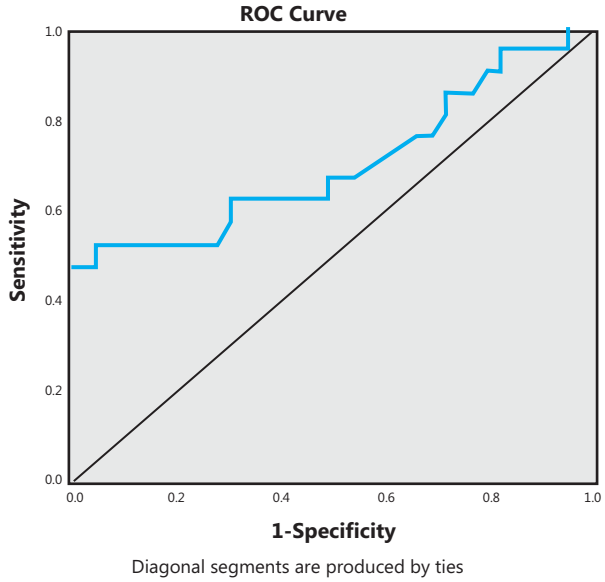

Figure 1. ROC curve for cut-off value of ureum

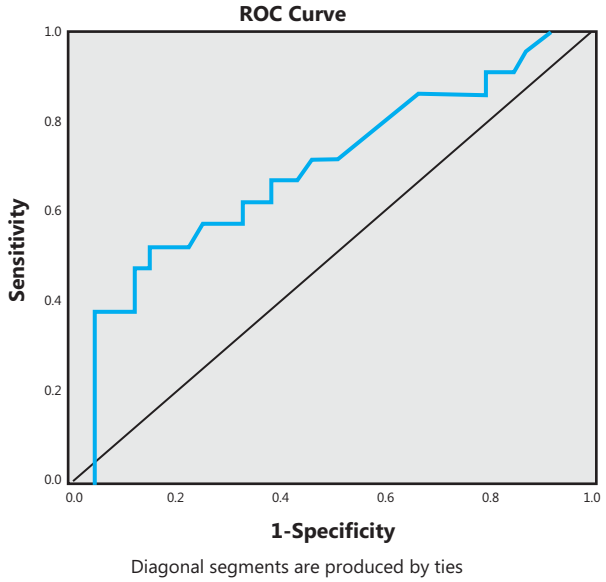

Figure 2. ROC curve for cut-off value of hs-CRP

Table 3. A 2x2 table between serum hsCRP, serum urea and other variable, which might affect total testosterone levels

Variable	Total Testosterone (ng/mL)		PR (95% CI)	p-value
	<3	≥3		
Age				
≥ 60 years	7	15	0.800 (0.263-2.435)	0.694
< 60 years	14	24		
Obesity				
Yes	10	7	4.156 (1.272-13.581)	0.015*
No	11	32		
hs-CRP (mg/L)				
≥ 0.65 mg/dL	13	12	3.656 (1.202-11.124)	0.020*
< 0.65 mg/dL	8	27		
Ureum (mg/dL)				
≥ 120 mg/dL	17	19	4.474 (1.273-15.728)	0.015*
< 120 mg/dL	4	20		
Hemodialysis				
≥ 3 months	15	37	0.135 (0.024-0.746)	0.018**
< 3 months	6	2		

Note: mg: milligram, L: liter, dL: desiliter, PR: Prevalence Ratio, CI: Confidence of Interval
*Pearson Chi-Square test, p<0.05 was significant
**Fisher exact test

Table 4. Logistic regression analysis of serum hs-CRP and others variables, which might affect total testosterone levels

Variable	PR	95% CI	p
Model 1			
Ureum ≥ 120 mg/dL	5.734	1.313-25.114	0.020*
Obesity	6.649	1.593-27.754	0.009*
hs-CRP ≥ 0.65 mg/dL	4.324	1.189-15.735	0.026*

Note: PR: Prevalence Ratio; CI: Confidence of Interval, hs-CRP: high sensitivity C-Reactive Protein. *p<0.05 was significant

Receptor (AR)-mediated lipolysis and inhibition of lipoprotein lipase activity in adipose tissue.[18,19]

This study showed a relationship between hs-CRP levels and total testosterone, with a PR of 3.235 (95% CI: 1.036-10.104; p=0.039) in stage V CKD patients. Proinflammatory cytokines such leptin, IL-1, IL-6 and TNF alpha are able to cause hypogonadism by impairing kisspeptin signaling, thereby reducing GnRH secretion. Decreased GnRH secretion is then followed by decreased LH secretion and decreased testosterone synthesis in Leydig cells. Stimulation of CRP synthesis mainly occurs in response to proinflammatory cytokines, especially IL-6 and TNF alpha, suggesting that plasma CRP levels reflect the number and activity of proinflammatory cytokines.[19] Similar results were also obtained in a study of Carrero *et al*. in male CKD patients with a total of 126 study subjects, which showed that there was a significant relationship between hs-CRP and total testosterone (p<0.0001).[20]

The loss of function of the kidneys to filter urea in CKD patients causes increased urea in the blood. This retention of toxic urea is the key to the mechanism of increasing the production of proinflammatory cytokines and Reactive Oxygen Species (ROS).[21] High levels of urea are able to block LH receptors on Leydig cells in the testes, making the testes unable to respond to LH to secrete testosterone.[22]

Bivariate analysis using Fisher's exact test showed a significant relationship between the duration of hemodialysis with total testosterone levels in this study with p=0.018. However, the PR value < 1 and the confidence interval range did not include the number one, indicating that the factor studied was not a risk factor, but rather a protective factor. Research by Li *et al*. suggested that patients on hemodialysis therapy for more than 8 months showed decreased urea and proinflammatory cytokines (CRP, IL-2 and TNF alpha) levels in the patient's serum (p<0.01). Continuous inflammation in CKD patients interferes with normal kidney function, resulting in a buildup of body metabolic waste. Hemodialysis aims to restore blood components to normal levels.[23]

The results of the multivariate logistic regression analysis in Table 4 and adjustment for urea 120 mg/dL, obesity and hs-CRP 0.65 mg/dL variables showed a significant relationship with total testosterone <3 ng/mL with p=0.020, p=0.009 and p=0.026, respectively. The urea 120 mg/dL showed a PR of 5.734 (95% CI: 1.31-25.114; p=0.020), obesity showed a PR of 6.649 (95% CI: 1.593-27.75; p=0.009) and hs-CRP 0.65 mg/dL showed a PR of 4.324 (95% CI: 1.189-15.735).

The use of cross-sectional design was one of the limitations in this study, making it unable to confirm a causal relationship between hs-CRP, obesity, urea levels and other variables with the decreased total testosterone levels in stage V CKD patients. In addition, gold standard LCMS was not used for the measurement of total testosterone levels in this study, this study was unable to determine the total testosterone levels before the patient was diagnosed with stage V CKD due to the inexistence of the symptoms or previous measurement of total testosterone levels, and there was no analysis of length of CKD in this study.

CONCLUSIONS AND SUGGESTIONS

This study showed that there was a statistically significant relationship between hs-CRP, serum urea, and obesity with decreased total testosterone levels in stage V CKD patients (p=0.020; p=0.015; and p=0.015) with cut-off urea of 120 mg/dL and hs-CRP 0.65 mg/dL with decreased total testosterone levels in stage V CKD patients. There was no significant relationship between age variable with the decrease in total testosterone levels (p=0.694). However, there was a significant relationship between the duration of hemodialysis with PR < 1 and the range of CI, which did not include number one, indicating that the factor studied was a protective factor, not a risk factor. It was necessary to perform further research using a cohort study design in order to obtain a hazard ratio to clarify the relationship among variables.

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