

Correlation between Serum Ferritin, Serum Cystatin C, and Renal Function in Children with β Thalassemia Major

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Abstract

Renal dysfunction caused by iron overload is characterized by an increase in ferritin and cystatin C levels. The objective of this study was to determine the correlation between ferritin, cystatin C, and renal function in children with β thalassemia major. A cross-sectional observational analytic study was conducted in September 2018 on 34 children with β thalassemia major in Dr. Hasan Sadikin General Hospital Bandung. Ferritin and cystatin C levels were documented and the estimated glomerular filtration rate (eGFR) was calculated using the Schwartz formula. Statistical tests were performed using Rank Spearman and Point Biserial with p value of <0.05 considered significant. the median ferritin level, cystatin C level, and eGFR of the subjects were 2,818 ng/mL (95% CI: 2,505–3,977), 209.9±121.5 (95% CI: 167.5–252.3), and 185.5 mL/min /1.73 m² (95% CI: 173.6–208.2), respectively. Correlations were sought between serum ferritin and eGFR (r=0.132, p=0.229), between cystatin C and eGFR: r=0.3012, p=0.041, and between ferritin and cystatin C: r=0.433, p=0.011. No correlation was found between ferritin and renal function whereas serum cystatin C presented a positive correlation with renal function. A strong correlation was found between ferritin and cystatin C. Serum ferritin and cystatin C are promising biomarkers to assist in monitoring renal function in children with β thalassemia major.

Key words: Cystatin C, ferritin, renal function, β talasemia major children

Hubungan Ferritin dan Cystatin C Serum dengan Fungsi Ginjal pada Anak Talasemia β Mayor

Abstrak

Kelebihan besi pada anak talasemia β mayor mengganggu organ vital di antaranya ginjal. Gangguan fungsi ginjal karena kelebihan besi dapat ditandai dengan peningkatan kadar ferritin dan *cystatin C*. Penelitian ini bertujuan mengetahui hubungan kadar feritin dan *cystatin C* dengan fungsi ginjal pada anak talasemia β mayor. Penelitian observasional analitik dengan rancangan potong lintang, dilaksanakan bulan September 2018. Subjek penelitian adalah anak penderita talasemia β mayor di Rumah Sakit Dr. Hasan Sadikin Bandung. Dilakukan pemeriksaan kadar ferritin dan *cystatin C*. Penilaian fungsi ginjal menggunakan *estimated glomerular filtration rate* (eGFR) dengan formula Schwartz. Uji statistik menggunakan uji Rank Spearman dan Point Biserial dengan kemaknaan berdasar nilai p<0,05. Didapatkan jumlah sampel sebanyak 34 anak, dengan kadar ferritin median 2818 ng/mL (IK95%:2505–3977), *cystatin C* 209,9±121,5 (IK95%:167,5–252,3) dan eGFR median 185,5 mL/menit per 1,73 m²(IK95%:173,6–208,2). Korelasi antara kadar ferritin dengan eGFR (r=0,132; p=0,229), korelasi *cystatin C* dengan eGFR (r=0,3012; p=0,041) dan korelasi kadar ferritin dengan *cystatin C* (r=0,433; p=0,011). Hasil penelitian ini tidak didapatkan korelasi antara feritin dan fungsi ginjal, sedangkan *cystatin C* memiliki korelasi positif dengan fungsi ginjal. Didapatkan korelasi kuat antara kadar ferritin dan *cystatin C* serum. Pemeriksaan kadar ferritin dan *cystatin C* dapat membantu pemantauan fungsi ginjal pada anak talasemia β mayor.

Kata kunci: Anak talasemia β mayor, cystatin c, ferritin, fungsi ginjal

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Introduction

Thalassemia is the most common inherited hemoglobinopathies in the world.¹ World Health Organization (WHO) stated that this hemoglobin abnormality causes a serious health problem in 229 countries and Indonesia is one of the countries with the highest number of thalassemia patients. Inadequate attention has been paid to renal dysfunction in children with β thalassemia since previous studies mostly only focused on adult patients when subclinical renal dysfunction is more common and could start earlier in thalassemia patients. Because red blood cell transfusion is the main treatment for thalassemia, iron excess may happen due to iron accumulation from repetitive blood transfusion, hemolysis, and body's compensation in the form of accelerated iron absorption caused by ineffective erythropoiesis. Ferritin serum testing nowadays are widely performed as an easy and trusted method to check iron concentration in the body.²⁻⁵

Iron excess in β thalassemia major is believed lead to various organ systems disruptions, such as disruptions in the heart, lungs, liver, endocrine glands, and, also, kidneys. Renal dysfunction can also be caused by previous anemia history, chronic hypoxia, or as the complication of iron chelation therapy. Poor chelation is an important risk factor for early renal affection.^{6,7} Renal dysfunction in β thalassemia major can be found in glomerulus or tubules and the parameter used to determine renal function is the glomerular filtration rate (GFR). The increasing level of ferritin serum will lead to GFR decline since iron excess damage renal's glomerulus and tubules.³ Endogen filtration marker test, such as serum creatinine and cystatin C, can be used in daily clinical practice since it is considered more convenient than the inulin test. Cystatin C level is closer to the GFR than the creatinine level.⁶⁻⁹

This study was performed to determine the correlation between serum ferritin, serum cystatin C, and renal function in children with β thalassemia major.

Methods

This was a cross-sectional analytical observational study on β thalassemia major children, selected consecutively. The sample size was determined using the sample size formula for correlation coefficient and a minimum of 34 subjects were required based on the formula. The exclusion

criteria was β thalassemia major children with systemic dysfunction/s (heart failure, liver failure, or diabetes mellitus) and who had been proven to experience renal dysfunction, both clinically and based on laboratory results. This study was performed in pediatric thalassemia outpatient ward in Dr. Hasan Sadikin General Hospital Bandung, Indonesia during the month of September 2018 after obtaining the approval from the hospital's Ethical Committee through the issuance of the ethical clearance number LB.04.01/A05/EC/244/VIII/2018. The parents of subject study had consented to their child's participation in the study, which was proven by signing the informed consent form. Anamnesis and physical examination were performed on the subjects to eliminate systemic abnormalities and anthropometric examination using WHO Growth Chart Standards (WCGS) curve was done and 5 mL blood sample was drawn from the peripheral venous. The blood samples were sent to the Pathological Clinic laboratory of the hospital to determine the ferritin serum level using Centaur XPT (Siemens). The determination of cystatin C serum level was performed using the human cystatin C immunosorbent assay (ELISA) kit added with hemoglobin level measurement using Sysmex complete blood count analyzer and creatinine serum measurement using Jaffe method (Siemens, 2016-12 version, USA).

The GFR assessment was performed using estimated Glomerular Filtration Rate (eGFR) followed with the use of Schwartz formula- eGFR for children ($1.73 \text{ m}^2/\text{mL}/\text{minute}$) = body height (cm) x constant number/serum creatinine (mg/dL), with the constant number of 0.44 for children <2 years old and 0.55 for children >2 years old. Patients were said to suffer from renal dysfunction if the GFR value was <90 mL/minute/ 1.73 m^2 . A persistent reduction in the GFR of <60 mL/minute/ 1.73 m^2 was defined as chronic kidney disease. The interpretation of GFR estimates depend on the clinical context. Patients with renal damage markers, such as proteinuria or abnormalities on imaging studies or presence of diseases on kidney biopsy, were considered to suffer from renal dysfunction even if the GFR estimates were 60 mL/minute/ 1.73 m^2 or greater.¹⁰

Data obtained were then recorded in SPSS® software for windows in version 25. Independent variable in this study was serum ferritin and cystatin levels while the dependent variable was the GFR. The correlation was analyzed using Rank Spearman Correlation for numerical data if data was not normally distributed and Point

Table Subject Characteristics

Variable	Normal value	n (%) n=34	Mean \pm SD	CI 95%
			Or Median (IQR)	
Age (years)	-		9 \pm 4	7-10
Gender	-			
Male		22 (64.7)		
Female	-	12 (35.3)		
Body weight (kg)			21.2 \pm 7.2	18.7-23.7
Body height (cm)			115.4 \pm 18.5	108.9-121.9
Nutritional status				
Good		2 (5.9)		
Malnourished		32 (94.1)		
Hemoglobin (g/dL)	11.5-14.5	6.9 \pm 1.3	6.5-7.4	20.9-26.7
Creatinine (mg/dL)	0.03-0.88		0.36 \pm 0.09	0.33-0.39
Ferritin (ng/mL)	10-300		2818 (2662)	2502-3977
Cystatin C (ng/mL)	580-1380		209.9 \pm 121.5	167.5-252.3
eGFR(mL/minutes/1,73 m ²)	<90		185.5 (50.3)	173.6-208.2

Note: SD=standard deviation, IQR=inter quartile range, CI=confidence interval

Biserial Correlation was used to determine the correlation between categorical and numerical data. Correlation strength value was determined as 0.000-0.1999 (very weak), 0.200-0.399 (weak), 0.400-0.599 (moderate), 0.600-0.799 (strong), 0.800-1.000 (very strong). The p value for significance was <0.05.

nutritional status, hemoglobin level, creatinine, ferritin, cystatin C, and eGFR as listed in Table. The correlation between serum ferritin and cystatin C was also analyzed and a significant correlation was identified (r=0.433, p=0.011), meaning that the higher the serum ferritin level is, the higher serum cystatin C level.

Results

The characteristics data of the subjects in this study include data on age, gender,

Discussion

Quality of life improvement in β thalassemia major children creates a long-term iron excess

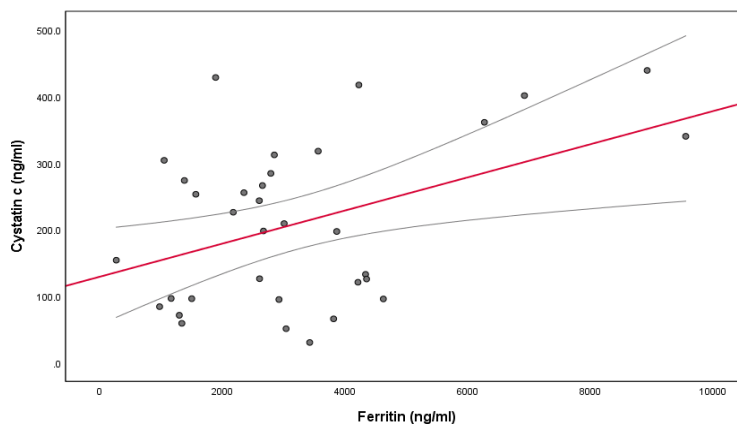


Figure 1 Scatterplot Fort Ferritin against Cystatin C

effect in vital organs such as liver, pancreas, heart, and kidneys. Renal dysfunction could be asymptomatic long before severe manifestations appear. Renal dysfunction is mostly multifactorial, possibly caused by previous anemia history, chronic hypoxia, iron excess, and iron chelation therapy.¹¹

This study found no correlation between serum ferritin level and GFR. High serum ferritin levels can reflect the condition of excess iron in the body, which is one of the causes of impaired renal function in patients with thalassemia β major.^{3,5} The median serum ferritin level in this study was 2,818 ng/dL, which was higher than the normal value. One study stated that the average serum ferritin value is different in each country.⁵ A study in Egypt regarding the examination of cystatin C as an early biomarker of renal abnormalities in adult patients with β thalassemia major has found an increase in serum ferritin level accompanied by a decrease in GFR.¹ Another study in Canada on renal dysfunction in thalassemia sufferers found no correlation between serum ferritin level and impaired renal function.¹² The lack of correlation between ferritin and GFR could be caused by a relatively increased GFR in the present study, caused by the overestimated GFR value due to malnourishment in 32 subjects (94.1%). Low creatinine level was observed in this study (0.36 ± 0.09 mg/dL) that may relate to the malnourished condition. Low creatinine level is triggered by low muscle mass and low creatinine production in malnourished children and this may lead to overestimated GFR value which cannot corrected with lower body height in malnourished children. Since malnourished children tend to have lower creatinine level, Schwartz formula should not be used for malnourished children.¹³

Cystatin C serum analysis showed a positive correlation with GFR. A previous study in Egypt regarding the examination of cystatin C as an early biomarker of renal abnormalities in adult patients with β thalassemia major obtained a negative correlation between serum cystatin C and GFR.¹ Examination of cystatin C was said to be more sensitive in assessing renal abnormalities compared to serum creatinine, because serum cystatin C can detect a small decrease in GFR.^{9,10} In this study, serum cystatin C level was normal and the GFR value relatively increased in all subjects. This is similar to the finding in a study in Iran on renal function in various types of thalassemia patients and a study in Canada about renal dysfunction in thalassemia patients.^{12,14} This condition occurs because of glomerular

hyperfiltration that is caused by anemia that a decrease in systemic resistance of blood vessels occurs, causing hyperdynamic circulation that will increase blood flow to the kidneys, resulting in an increase in GFR.¹⁵⁻¹⁷ The study concluded that renal function disorders do not occurred in all subjects but the GFR will decrease with age, especially in patients who do not routinely receive transfusions. In the long term, chronic hypoxia can cause a progressive decrease in GFR, due to apoptosis and tubular interstitial damage, until glomerular sclerosis and renal fibrosis occur. Studies in Italy on the long-term follow-up of renal function in β thalassemia major patients stated that a progressive decline will occur at the age of 20–30 years.^{4,18,19} This situation is aggravated by the excess iron which causes damages to glomerular cells and tubules. These damaged cells then secrete cytokines and growth factors that can also trigger glomerular and tubular sclerosis.¹⁷

A significant correlation between serum ferritin level and serum cystatin C levels was found in this study with the higher the serum ferritin level is, the higher the serum cystatin C level. This increased serum ferritin that is accompanied by an increase in serum cystatin C is similar to the finding in Egypt that suggested cystatin C as a good marker to help monitor renal dysfunction from small changes in GFR with higher sensitivity and specificity than creatinine. In their conclusion, they mentioned that renal dysfunction in β thalassemia major is not rare complication that markers such as cystatin C are useful for early detection of small changes in GFR.^{1,6,20}

There are limitations in our study due to its design as a cross-sectional study that it is not possible to follow the β thalassemia major patients over time.

In conclusion, a strong correlation between serum ferritin level and serum cystatin C level is observed. Serum ferritin and cystatin C are promising biomarkers to assist in monitoring renal function in children with β thalassemia major.

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