THE NEW ARMENIAN MEDICAL JOURNAL
Vol.13 (2019), No 4, p. 95-99

RELATIONSHIP BETWEEN SERUM IRON, TOTAL IRON BINDING CAPACITY, TRANSFERRIN SATURATION AND HEPCIDIN IN PATIENTS WITH STAGE 3 - 5 NON-DIALYSIS CHRONIC KIDNEY DISEASE

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Received 15.12.2018; accepted for printing 15.10.2019

Abstract

Background: Functional iron deficiency anemia is one of the complications that often occurs in patients with chronic kidney disease. Imbalance of hepcidin can induce iron disorder which is the pathophysiology of anemia in chronic kidney disease. Measurement of serum iron, total iron binding capacity and transferrin saturation aims to determine the availability of iron in the circulation. This study determines the relationship between serum iron, total iron binding capacity, transferrin saturation and hepcidin in non-dialysis chronic kidney disease.

Objective: Determining the relationship between serum iron, total iron binding capacity, transferrin saturation and hepcidin in stage 3 - 5 non-dialysis chronic kidney disease.

Methods: This cross-sectional analytic observational study evaluates 37 stage 3-5 non-dialysis chronic kidney disease patients whose age between 20 to 80 years old in outpatient nephrology clinic of Dr. Soetomo Hospital. Serum iron, total iron binding capacity, transferrin and hepcidin saturation levels are measured. This study is done by consecutive sampling and spearman parametric test is used to analyze the data.

Results: Of the 37 subjects, 67.6% are men with mean age is 56.84 ± 12.27 years. Most patients in this study is in stage 3. The mean hemoglobin, serum creatinine, serum iron and total iron binding capacity levels in the subjects of this study are 10.88 ± 2.21 g/dl, 3.42 ± 2.25 mg/dl, 64.86 ± 24.15 mg/dl and 235.1 ± 67.45 ng/dl consecutively. Median transferin saturation and hepcidin levels are 27% (4 – 88%) and 48.58 mg/dl (0.12 - 439.07 ng/ml). There is a relationship between total iron binding capacity and hepcidin with negative correlation coefficient of - 0.72 (p = 0.000), but there is no relationship between serum iron and transferrin saturation with hepcidin.

Conclusion: In this study, there is a significant negative relationship with moderate strength between total iron binding capacity and hepcidin. The r value is -0.725 with p = 0.000.

Keywords: *anemia, chronic kidney disease, hepcidin, serum iron, total iron binding capacity, transferrin saturation*

Introduction

Anemia that often occurs in chronic kidney disease (CKD) is functional iron deficiency anemia. Measurement of iron status is needed to diagnose and treat this type of anemia [*Cullis J, 2013, Atkinson MA et al., 2015*]. Iron status in functional iron deficiency anemia usually causes an increase in ferritin and decrease in circulating iron. In recent year, hepcidin which acts as a regulator of iron balance becomes the main pathophysiology of functional iron deficiency anemia. Hepcidin production will increase if there is inflammation or acute conditions [*Drakesmith H, Prentice AM, 2012; Cullis J, 2013*]. Chronic inflammation that occurs in CKD increases

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hepcidin production. Hepcidin main function is to inhibit the absorption of iron in the intestine and block ferroportin which is the entrance and exit of iron in macrophages. This mechanism causes an iron to decrease [*Coyne DW, 2011; Arezes J, Nemeth E, 2015; Fraenkel PG, 2015*]. Clinicians usually perform serum iron, total iron binding capacity, and transferrin saturation tests to see the decrease the iron's availability in the circulation [*Coyne DW, 2011, Arezes J, Nemeth E, 2015*]. In this study, we will determine the relationship between serum iron, total iron binding capacity, and transferrin saturation with hepcidin in patients with CKD stage 3-5 non-dialysis.

Materials and Methods

The population were 20-80 years old male and female patients who are diagnosed with stage 3 - 5 non-dialysis CKD in the renal and hypertension clinic of Dr. Soetomo General Hospital Surabaya. Exclusion criteria are hepatitis B, hepatitis C, liver cirrhosis, diabetes mellitus, obesity, and patients receiving erythropoietin/ steroid therapy/ iron therapy/ blood transfusion/ hormonal therapy (within 3 months). This is a cross-sectional analytic observational study design. We collect data including age, gender, stage of CKD, causes of CKD, as well as biochemical parameters-hemoglobin (*g/dl*), serum iron (*mg/dk*), total iron binding capacity (*ng/dl*) , saturation of transferrin (*%*) and hepcidin (*ng/ml*). Biochemical parameters were determined in the laboratory PRODIA WIDYAHUSADA (www.prodia.co.id.) (Surabaya, Indonesia). This study was conducted in October-December 2017 by taking blood samples with a sequential sampling method to satisfy a minimum sample of 37 samples. The data were analyzed using the Pearson test with a value of p <0.005. The methodology is cross sectional and we presented the data using SPSS ed IX

Results

A total of 37 samples met the inclusion and exclusion criteria of this study. The general characteristics of this study can be seen in table 1. The demographic characteristics of this study show that male is 67.6% and female is 32.4%. This research sample age ranged from 18 to 80 years with mean age is 56.84 ± 12.27 years. The most common CKD stages in this study are stage 3 (43.2%), followed by stages 5 (32.4%) and 4 (24.3%).

The distribution of serum iron and total iron binding capacity has a normal data distribution whereas transferrin saturation and hepcidin has an abnormal data distribution. We use Spearman test

Table 1.

General Characteristics

Category	Frequency
Age	
Mean ± SD (Years old)	56.84 ± 12.27
Gender	
Male	25 (67.6%)
Female	12 (32.4%)
CKD Stage	
Stage 3	16 (43.2%)
Stage 4	9 (24.,3%)
Stage 5	12 (32.4%)
Biological Characteristics	
Hemoglobin (Mean ± SD) (*g/dl*)	10.88 ± 2.21
Creatinin Serum (Mean ± SD) (*mg/dl*)	3.42 ± 2.25
Serum Iron (Mean ± SD) (*mg/dk*)	64.86 ± 24.15
Total Iron Binding Capacity (Mean ± SD) (*ng/dl*)	235.1 ± 67.45
Transferin Saturation (Median) (*%*)	27 (4 – 88)
Hepcidin (Median) (*ng/ml*)	48.58 (0.12 - 439.07)

Table 2.

Spearman Test of serum iron, total iron binding capacity, Transferrin saturation and hepcidin

Variable	**R (p-value**	**Conclusion**
Serum Iron	- 0,45 (0,791)	No relationship
Total iron binding capacity	- 0,72 (0,000)	Significant relationship
Transferrin saturation	0,284 (0,088)	No relationship

to see the relationship. There is relationship between total iron binding capacity and hepcidin with negative correlation coefficient of - 0.72 (p = 0,000) but there is no relationship between serum iron and transferrin saturation with hepcidin. The result can be seen in table 2.

Discussion

The mean research subjects age is 56.84 ± 12.27 years. That result is almost the same with other comparative studies with an average age of 64.3 ± 16.9; 58.4 ± 14.8 years; and 68 years [*Zaritsky J et al., 2009; Troutt JS et al., 2013; Mercadal D et al., 2014*]. The average age is not different from comparative research, because the number and quality of nephrons will decrease in line with increasing age so the kidney function will decline faster [*Troutt JS et al., 2013*]. The subjects were mostly male (67.6%), this result is almost the same as the comparative study which is also mostly male with a percentage of 57.3% and 65% [*Mercadal D et al., 2014*]. The percentage of men who are diagnosed with CKD is higher than women, because of various factors involvement such as hypertension, hyperglycemia, lifestyle, kidney structure and hormonal differences [*Chang PY et al., 2016*]. Hemoglobin levels has an average value of 10.88 *g/dl* with the standard deviation of 2.21. The results of this study are different from the comparative studies conducted by [*Zaritsky J et al., 2009, Troutt JS et al., 2013, Mercadal L et al., 2014*] with mean hemoglobin is above 12 *g/dl*. These different results might happen because of unequal sampling. Other studies have included research subjects who are currently on iron therapy, erythropoietin, and blood transfusion. Also, the measurement of low hemoglobin in this study can be related to various factors such as decreased erythropoietin, increased hepcidin, the presence of uremic gastropathy, and lack of food intake [*Zaritsky J et al., 2009*]. The mean creatinine in this study is 3.42 ± 2.25 *mg/dl*. This result is slightly different from the comparative study which obtained a mean creatinine of 4.9 ± 0.3 *mg/dl* [*Troutt JS et al., 2013*]. This difference can be related to sample measurements in the laboratory with different measurement machines.

The mean serum iron in this study is 64.86 ± 24.15 *mg/ml*. The mean of the results of this study is almost the same as the mean of the results of a comparative study that is 56.85 ± 8.53 *mg/ml* and 69.4 ± 4.4 *mg/ml* [*Troutt JS et al., 2013*]. The results of this study mean serum iron levels are still within normal limits, this is related to samples that have an evenly distributed population between stages. Serum iron levels from the beginning of the CKD stage to the final stage will decrease due to the role of hepcidin in inhibiting the entry of iron into the circulation. Serum iron levels in the early stages are usually still within normal limits[*Jacobs C et al., 2005, Drakesmith H, Prentice AM, 2012, Cullis J, 2013*]. The mean total iron binding capacity in this study is 235.1 ± 67.45 *mg/ml*. The mean of the results of this study is almost the same as the mean of the results of a comparative study that is 256.6 ± 45.54 *mg/ml*. The median transferrin saturation in this study was 27% (4 – 88%). The results of this study is almost the same as the results of comparative studies in the amount of 23.2 ± 8.6% [*Zaritsky J et al., 2009*] 24.6 ± 4.8%, [*Jelic M et al., 2013*] 35.8 ± 13,7 [*Troutt JS et al., 2013*], 25.47 ± 9.83 [*Mercadal L et al., 2014*]. Transferrin saturation in CKD will mostly increase due to compensation for the availability of iron in the circulation. This occurs because of the inhibition of iron intake from intestinal absorption and macrophage output due to the role of hepcidin [*Jelic M et al., 2013*].

The median total hepcidin in this study is 48.58 *ng/ml* (0.12 - 439.07) (control value of hepcidin levels is 20.65 - 42.89). The median hepcidin levels that corresponds to CKD stage are at stage 3 : 23.17 *ng/ml* (range 0.12 - 70.14), stage 4 : 83.02 *ng/ml* (range 1.08 - 254.87), and at stage 5 : 118.86 *ng/ml* (range 9.96 - 439.07). This study is no different from comparative studies which concluded that there was an increase in hepcidin levels at each stage of CKD [*Zaritsky J et al., 2009, Mercadal et al., 2014, Manolov et al., 2015*]. The increase in hepcidin at each stage of CKD complies the theory that inflammation in CKD will increase the production of hepcidin in the liver as well as the disruption of hepcidin removal in the kidneys so hepcidin will accumulate in circulation [*Babitt JL, Lin HY, 2012; Wang C, Babitt JL, 2016*]. The

results of the relationship analysis between serum iron, total iron binding capacity and transferrin saturation with hepcidin in CKD patients show that only total iron binding capacity with hepcidin has a significant relationship (correlation coefficient of - 0.72; p-value of 0,000). The result of this study is consistent with the results of research conducted by Manolov et al. (2015) (-0.089 with p-value <0.05) and Mercadel et al. (2014) (r - 0.32 with p-value <0.0001) which shows a significant relationship between total iron binding capacity and hepcidin in patients with CKD [*Mercadal L et al., 2014, Manolov V et al., 2015*]. This relationship shows a discrepancy theory which states that with an increase of hepcidin there will be a barrier to ferroportin which results in the iron trapped inside the macrophages, and then decreasing the availability of iron in circulation. After that, body compensates by increasing transferrin and total iron binding capacity [*Babitt JL, Lin HY, 2012; Drakesmith H, Prentice AM, 2012*]. This study does not compare between the control group and the dialysis group. This study also does not analyze elevated levels of serum iron, total iron binding capacity, serum transferrin, and hepcidin at each stage of CKD.

The results of the relationship analysis between serum iron, total iron binding capacity and transferrin saturation with hepcidin in CKD patients show that only total iron binding capacity with hepcidin has a significant relationship (correlation coefficient of - 0.72; p-value of 0,000). The result of this study is consistent with the results of research conducted by Manolov et.al (2014) (-0.089 with p-value <0.05) and Mercadel et al. (2014) (r - 0.32 with p-value <0.0001) which shows a significant relationship between total iron binding capacity and hepcidin in patients with CKD [*Mercadal L et al., 2014, Manolov V et al., 2015*]. This relationship shows a discrepancy theory which states that with an increase of hepcidin there will be a barrier to ferroportin which results in the iron trapped inside the macrophages, and then decreasing the availability of iron in circulation. After that, body compensates by increasing transferrin and total iron binding capacity [*Babitt JL, Lin HY, 2012; Drakesmith H, Prentice AM, 2012*]. This study does not compare between the control group and the dialysis group. This study also does not analyze elevated levels of serum iron, total iron binding capacity, serum transferrin, and hepcidin at each stage of CKD.

CONCLUSION

There is a significant correlation with moderate strength between total iron binding capacity levels and hepcidin levels (r = - 0.72; p-value 0,000) which indicates if there is a decrease in total iron binding capacity levels, there will be an increase in hepcidin levels.

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