Role of hepcidin to identify the type of anemia in chronic kidney disease in children

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Abstract. Chronic kidney disease (CKD) may present with anemia of chronic disease (ACD), iron-deficiency anemia, or both (mixed anemia). Common hematologic parameters may not distinguish type of anemia in CKD. Hepcidin is a new variable considered to guide management of anemia in CKD. This study aimed to determine type of anemia in children with CKD, and determine the level of hepcidin in those patients and its relationship with degree of CKD, hemoglobin, and ferritin. This was a cross sectional study in 2-18 years non-dialyzed children with CKD. Subjects were divided into group I (CKD stage 1-2) and group II (CKD stage 3-5). Each group consisted of 29 subjects. Anemia occurred in 34 of 58 subjects, 24 were ACD and 10 were mixed anemia. Median of hepcidin levels in group II were significantly higher than group I (33.4 vs 12.5 ng/mL). Hepcidin has positive correlation with ferritin. ROC analysis showed that hepcidin level of >18 ng/mL may predict ACD. Ferritin level of >99.7 ng/dL can predict hepcidin >18ng/mL (sensitivity 74.2% and specificity 70.4%). This study concluded that ACD is the most type of anemia in CKD besides mixed anemia.

1. Introduction
Anemia is a common complication in chronic kidney disease (CKD) and contributes to an increased morbidity and mortality of the patients. This is mainly because anemia is a risk factor for cardiovascular disease, which is the leading cause of death CKD patients [1,2,3].

The prevalence of anemia in children with CKD varies around 36.6 %. The incidence of anemia increases with low glomerular filtration rate (GFR). The incidence according to stages of CKD is 31-57 % for stage I-II, 73 % for stage III, 87 % for stage IV, and 93.3 % for stage V [4,5,6]. In adults, the decrease of hemoglobin (Hb) level will only be visible at GFR <60mL/min/1.73m² [2,7,8]. This is slightly different in children, where an increase of hazard ratio (HR) for the occurrence of anemia is consistent with the decline in GFR [6].

The main cause of anemia in CKD is the decreased production of erythropoietin which is in line with the degree of impairment of renal function. Treatment with erythropoietin stimulating agents (ESA) could increase Hb level and improve cardiovascular function of most CKD patients, thus improving the prognosis of CKD [9]. However, some patients were hypo or non-responsive, as shown by the low level of Hb in more than 20% of children with advanced CKD despite administration of ESA [10,11,12].

The ESA hypo-responsiveness is commonly treated by increasing the dose of ESA (dose escalation). However, several clinical trials in adults have highlighted the side effects of ESA therapy,
such as that affecting the cardiovascular and cerebrovascular system as well as death [13]. The identification of ESA hypo/non-responsiveness is part of the management of anemia in CKD [11,14]. Although clinical trials in children are still limited, several studies have shown benefit of iron supplementation in patients who are hyporesponsive to ESA.

Iron-deficiency anemia (IDA) has been known as one of risk factors for ESA hyporesponsiveness in CKD. CKD patients are at risk of developing iron deficiency due to reduced iron intake caused by the loss of appetite and blood loss during hemodialysis. This condition is referred to as absolute iron deficiency, and can be treated by iron supplementation [10,15]. In CKD, iron deficiency can be caused by the increase of iron consumption for erythropoiesis when given ESA. This condition is known as functional iron deficiency or relative iron deficiency [11,16].

High levels of ferritin and Hb are found in anemia of chronic disease (ACD). This is mainly caused by the abnormality of iron homeostasis as the result of iron intake and retention in the reticuloendothelial system (iron sequestration syndrome). The iron intake reduce the level of iron in the circulation thereby diminishing its availability for eritroid progenitors (functional iron deficiency) [10,15].

Anemia of chronic disease as well as absolute and functional iron deficiency are conditions that can inhibit erythropoiesis (iron-restricted erythropoiesis - IRE). Differentiating between those conditions is quite complicated [15]. The absolute iron deficiency can be easily recognized from low levels of Hb, serum iron, ferritin dan transferrin saturation. The functional iron deficiency is diagnosed from the normal level of transferrin with normal or slightly increased level of ferritin. Meanwhile, the iron deficiency on chronic disease is recognized from the low level of serum iron and transferrin saturation accompanied by an increase of ferritin. The level of ferritin and transferrin are influenced by inflammation which complicates the interpretation [10].

The limitation in the current parameters for iron deficiency has prompted the search for better parameters. Previous research has shown that soluble transferrin receptor (sTFR) is indeed able to differentiate between IDA from ACD because it is not influenced by inflammation. The increased level of sTFR is observed in the iron deficiency state, but not in chronic diseases. However, the interpretation of the sTFR level can be difficult in CKD, where both functional iron deficiency and anemia of chronic disease are present due to the iron retention [10,17].

Hepcidin, an acute phase protein in iron regulation, can provide information regarding the immune response, iron homeostasis and anemia in chronic disease. Several studies have shown the correlation between hepcidin and ferritin [6,10,16,18]. Hepcidin is more superior in describing iron status, iron availability and erythropoiesis compared to ferritin. Therefore, hepcidin is a potential marker for iron status, particularly in ESA therapy [6,15,17,18,19]. The aim of this study was to determine the cause of anemia in children with CKD, and measure the level of hepcidin level. This study also analyzed the correlation of hepcidin level with GFR, Hb level and ferritin.

2. Methods
This research is a cross sectional study conducted at the Department of Pediatrics at Dr. Cipto Mangunkusumo (RSCM) Jakarta and RSUP Dr. Mohammad Hoesin (RSMH) Palembang between April-October 2015. This study was approved by the Ethical Committee of the Faculty of Medicine, Universitas Indonesia.

The subjects were consecutively selected from children with CKD between the age of 2-18 years old. CKD was determined from the kidney abnormality detected from laboratory results, imaging or histopathology; or GFR < 60 mL/min/1.73m², which were evident for more than three months. The GFR was calculated with the Schwartz formula. Patients with hemolytic anemia, bleeding, malignancy, osteodystrophy, infection (leukocytosis or CRP ≥ 12 mg/dL or CRP positive), and ferritin level <30 ng/dL or >1000 ng/dL, were excluded. Patients who had kidney replacement therapy ≤ 6 months before recruitment, or erythropoietin therapy ≤ 2 weeks before recruitment, or blood transfusion ≤ 4 months before recruitment were excluded. The variables measured were Hb, ferritin, sTFR dan hepcidin levels. A written informed consent was obtained before recruitment. Venous blood (6 mL) was collected for laboratory analysis in Prodia Laboratory, Kramat Jakarta Pusat using Quantikine IVD Human sTFR immunoassay kit and DRG hepcidin-25 (bioactive) ELISA kit.
Subjects were grouped into two groups, Group I (CKD stage I-II or GFR >60mL/min/1,73m²) and Group II (CKD stage 3-5 or GFR≤60mL/min/1,73m²). The anemic state was classified into anemia of chronic disease (ferritin level ≥ 100 ng/dL, sTFR <2,7 mg/L and/or sTFR/log ferritin index <1,5), and mixed anemia (ferritin level <100 ng/dL, sTFR >2,7 mg/L and/or sTFR/log ferritin index >1,5).

Data were analyzed using SPSS v20. The normality test was conducted for all numerical data with Kolmogorov-Smirnov test for sample size more than 50, or Shapiro-Wilk test for sample size less than 50. Normally distributed data is presented as mean ± standard deviation (SD), while skewedly distributed data is presented as median (range). The bivariate analysis for numerical data was analyzed using independent T-test, or Mann-Whitney test for skewed distribution. Categorical data was analyzed with chi-square test. Correlation analysis was performed using Pearson test, or Spearman test for skewed data. The sensitivity and specificity and cut-off point were determined with ROC curve and AUC. A p value < 0,05 is considered to be statistically significant.

3. Results
Out of 75 eligible participants, 48 subjects were from RSCM Jakarta and 27 were from RSMH Palembang. Seventeen subjects were excluded, 11 subjects excluded due to ferritin level <30 ng/dL, 3 subjects had ferritin >1000 ng/dL, 2 subjects were obese and 1 subject had positive CRP. Therefore, a total of only 58 subjects were included into analysis. Subjects were divided into Group I (CKD stage I-II or GFR >60 mL/min/1,73m²) and Group II (CKD stage 3-5 or GFR≤60 mL/min/1,73m²). The mean age of group I and group II were 124 months (SD 48,3) and 134 months (SD 46,4). Age, sex, and nutritional status between two groups were not significantly different. The etiologies of CKD in this study were chronic glomerulonephritis (n=19), nephrotic syndrome (n=16), obstructive nephropathy (n=16), neurogenic bladder (n=7), and renal hypoplasia (n=5).

Table 1 shows the laboratory result between two groups. Mean Hb level is significantly higher in group I compared to group II with mean difference of 3,3 g/dL (95% CI 2,41-4,24). Ferritin level in group II is higher than group I, but is not statistically significant. The mean hepcidin level in group II is significantly higher than group I, with mean difference of 30,4 ng/mL (95% CI 18,9-41,9). The correlation test shows strong negative correlation between GFR and hepcidin (R= -0,608). This means that the decrease in GFR is followed by increase in hepcidin level (p<0.001). Hepcidin level positively correlates with ferritin (R=0,513), meaning that hepcidin level will increase with the increase of ferritin (p<0.001).

| Laboratory parameter | Group I (n=29) | Group II (n=29) | p   |
|----------------------|----------------|-----------------|-----|
| Hb (g/dL)a           | 12,7 ± 1,47    | 9,3 ± 1,97      | <0,001 |
| Ferritin (ng/dL)b    | 70 (30,7-867)  | 183 (33-642)    | 0,068 |
| Hepcidin (ng/mL)b    | 12,5 (0,35-34,62) | 33,4 (13,76-135,15) | <0,001 |

aMean ± SD, T-test;  
bMedian(range), Mann-Whitney test

Anemia was observed in 34 out of 58 subjects, specifically 7/29 subjects in group I, and 27/29 subjects in group II. The incidence of anemia is significantly higher in group II compared to group I with OR 4,452. Low level of Hb had a positive correlation with reduced GFR (R=0,728).

Subjects with anemia (n=34) had mean hepcidin level of 35,9 ng/mL (SD 30,6) with median 31,2 ng/mL (range 0,35-135). Hepcidin level in those without anemia (n=24) was 13,6 ng/mL (SD 6,82) with median 12,8 ng/mL (range 0,35-28,81). The mean hepcidin level of anemic subjects was significantly higher than non-anemic subjects with mean difference of 24,3 ng/mL (95% CI 11,6-37,1; p<0.001).
Out of 34 anemic subjects, 24 subjects were classified as ACD and 10 were as mixed anemia. ACD was the major cause of anemia in both groups, 4/7 subjects and 20/27 for group I and II, respectively. Mixed anemia was the cause for 3/7 and 7/20 in group I and II, respectively. The mean hepcidin level for ACD (n=24) is 47.08 ng/mL (SD 31.92) with median 36.5 ng/mL (range 0.35-135.15), while the hepcidin level for mixed anemia (n=10) is 15.96 ng/mL (SD 8.41) with median 17.5 ng/mL (range 0.35-29.72). The mean hepcidin level for ACD is significantly higher in ACD than mixed anemia with mean difference of 31.11 (95% CI 10.0-52.1; p<0.001).

Hepcidin level was higher in ACD compare to mixed anemia in both groups, as seen in Figure 1. Subjects in group I with ACD (n=4) have median hepcidin level of 14.08 (range 0.35-34.62), while subjects with mixed anemia (n=3) have median hepcidin level of 4.5 (range 0.35-17.96). The hepcidin level in ACD was higher than mixed anemia, with mean difference of 8.2 ng/mL (95% CI 4.4-70.7), however it is not statistically significant. In group II, subjects with ACD (n=20) have median hepcidin level of 30.88 (range 17.11-135.15), subjects with mixed anemia (n=7) have median hepcidin level of 19.9 (range 13.76-29.72) with mean difference of 33.7 (95% CI 9.3-58.2). The hepcidin level in ACD in group II is significantly higher than mixed anemia (p 0.001).

Figure 1. The distribution of hepcidin level based CKD stage and type of anemia

Out of 24 subjects without anemia, there were 6 subjects (5 subjects of group I and 1 subject of group II) with serum ferritin level<100 ng/dl and sTFR >2.7 mg/L and/or sTFR index >1.5 showing that these subjects have functional iron deficiency with no apparent anemia. The mean hepcidin level in these subjects is 14.12 ng/mL, median 13.4 ng/mL (range 9.0-20.9).

Figure 2 shows an ROC analysis that hepcidin is a better parameter to predict ACD compared to ferritin with AUC 0.854 and 0.837, respectively. The hepcidin cut-off point >18 ng/mL can predict ACD with sensitivity and specificity of 83.3% and 60.0%, respectively.

ROC analysis was conducted to obtain whether ferritin can be used predict hepcidin level. ROC analysis showed AUC 0.786, meaning that ferritin is a good parameter to predict hepcidin level (Figure 3). Ferritin with cut-off point of >99.7ng/dL can predict hepcidin level >18ng/mL with sensitivity and specificity of 74.2% and 70.4%, respectively.
Figure 2. ROC analysis and AUC value for hepcidin, ferritin and Hb value to predict anemia of chronic diseases

Figure 3. ROC curve for ferritin to predict hepcidin level

4. Discussions

The result shows that the prevalence of anemia is statistically higher in group I (CKD stage I-II or GFR >60mL/min/1,73m²) compared to group II (CKD stage 3-5 or GFR≤60mL/min/1,73m²). Similar to previous studies, the decrease in GFR was followed by the decrease in level of Hb [2,4-8]. In contrast, the mean level of ferritin in group II was higher than group I, although not statistically significant (p=0,068). This finding is similar to Zaritsky et al. who also observed a higher mean of ferritin in advanced stages of CKD [20].

Anemia was observed in 34 of 58 subjects, with a total of 27 subjects had ACD, and 7 subjects had mixed anemia. Anemia in CKD is mainly caused by ACD or anemia of inflammation (AoI). In this condition, the level of hepcidin is usually increased and can cause retention of iron reserve followed by a decrease in intestinal iron absorption. These circumstances diminish the iron supply for erythropoiesis (iron restricted erythropoiesis), despite the availability of iron reserve (the increase in ferritin level). By contrast, in absolute iron deficiency, the low level of hepcidin is meant to promote intestinal iron absorption and release the iron reserve, to increase iron supply for erythropoiesis [11].

The mean hepcidin level in group II is statistically higher than group II. Zaritsky et al. reported that the median hepcidin level increased to 652,4 ng/mL in CKD stage 5D, 127,3 ng/mL in CKD Stage 2-4 and 25,3 ng/mL in control group [20]. These showed that the main cause of anemia in higher stages of CKD is associated with higher level of hepcidin. The level of hepcidin in this study is lower to that of Zaritsky et al., likely because of exclusion of dialyzed patients [21].

In the earlier stage of CKD, the mean hepcidin level is lower (median 12,5 ng/dL) compared the normal mean Hb level. However, according to Choi et al., the mean hepcidin level in early stage CKD is still higher than the median of normal adult Hb level in the population (7,8 ng/mL) with the cut-off point of 6,895 ng/mL for iron deficiency in children [22,23].

The ROC analysis was conducted to obtain the cut-off point of the parameter in predicting ACD as the main cause of anemia. Hepcidin is a better parameter to determine ACD compared to ferritin, based on AUC value in ROC analysis (figure 2). The cut-off point of hepcidin (>18 ng/mL) can be
used to predict ACD with the sensitivity and specificity of 83.3% and 60%, respectively. ACD with high hepcidin level will result in high ferritin level due to the iron retention [10,11].

Hepcidin measurement is not a common laboratory measurement. The hepcidin level has positive correlation with the ferritin level (R=0.513), which means that the increase in the ferritin correlates with the increase of hepcidin level (p<0.001). This study showed that ferritin can be used to predict the level of hepcidin (figure 3). The cut-off point of ferritin (>99.7 ng/dL) can predict the hepcidin level (>18.0 ng/mL) with sensitivity of 74.2% and specificity of 70.4%. This result supports KDOQI recommendation to consider cut-off ferritin level of 100 ng/dL [5,10].

Intravenous iron supplementation is required for anemic subjects with high hepcidin level (>18 ng/mL), particularly those with CKD stage 3-5, since oral iron absorption is reduced due to the high hepcidin level. Further optimization of ESA dosage is required in order to decrease the hepcidin level [10,11,24].

Although the major cause of anemia in this study was ACD, we still have to be aware of mixed anemia because it consists of one third of anemia in CKD, particularly in subjects with lower GFR. The average hepcidin level in subjects with mixed anemia was 17.5 ng/mL (range 0.35-29.72), and was slightly higher (19.9) in subjects with lower GFR (group II) (range 13.76-29.72). Both hepcidin levels were higher compared to the median in subjects without anemia 12.8 ng/mL (range 0.35-28.81). Iron retention could also exist in this condition, thereby causing us to be aware that our oral iron supplementation might not be absorbed properly due to the high level of hepcidin [10,11].

This study recommends further exploration for anemia in CKD, particularly in patients with no or less response to the standard therapy. The measurement of hepcidin level is highly recommended in those conditions.

5. Conclusions
ACD is the major cause of anemia in CKD. The decrease in GFR is accompanied by the decrease of Hb level as well as the increase of ferritin and hepcidin level. The hepcidin level in CKD stage 3-5 is higher than that of subjects with earlier stages of CKD. The hepcidin level >18 ng/mL is a good parameter to predict the cause of anemia is ACD (sensitivity 83.3% and specificity 60.0%). Because the measurement of hepcidin is not routinely available, ferritin level of >99.7 ng/dL can be used to predict hepcidin level of >18 ng/mL (sensitivity 74.2% dan spesificity 70.4%).

6. References
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