The Role of Vitamin D and Hepcidin in Pathophysiology of Anaemia in Children with Chronic Kidney Disease

Hertanti Indah Lestari1,2*

1Department of Pediatric, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia
2Dr Mohammad Hoesin General Hospital, Palembang, Indonesia
*Correspondence author email: hertantiindah@gmail.com

ABSTRACT

In patients with Chronic Kidney Disease (CKD), renal function in maintaining the homeostasis of various metabolic systems of the body is impaired, one of which is the production of erythropoietin hormones. Anaemia is a frequent complication of the CKD and contributes to the increased morbidity and mortality of the patients. Diagnosis or treatment of anaemia in PGK is still a challenge due to multifactorial. Hepcidin, a peptide that has been known as an acute-phase protein, plays a vital role in the regulation of iron metabolism. Hepcidin is known to correlate with ferritin, a parameter of anaemia screening which is commonly examined during this time. In addition to the role of pathophysiology anaemia in CKD, hepcidin is currently widely researched because it is suspected to contribute also to the pathophysiology of bone mineral disorders in PGK. Current research shows that the concentration of vitamin D is inversely proportional to the concentration of hepcidin and is positively associated with the concentration of haemoglobin and iron. The latest report invitro and in vivo support the existence of the interaction between vitamin D and anaemia in patients with PGK through the role of hepcidin.

Keywords: anemia, hepcidins, vitamin D, erythropoietin, chronic renal insufficiency.
INTRODUCTION

The kidneys play an essential role in the homeostasis of various systems and metabolism in the body. In patients with chronic kidney disease (CKD), kidney function in maintaining the homeostasis of various metabolic systems in the body is disturbed, which can cause severe and life-threatening complications. One of the essential functions of the kidneys is that the kidneys produce the hormone erythropoietin, so it also plays a role in the formation of red blood cells (erythropoiesis). Lack of red blood cells (anaemia) is a complication that often occurs in chronic kidney disease (CKD) and plays a role in increasing the morbidity and mortality of CKD sufferers. Anaemia is a risk factor for the incidence of cardiovascular disease, which is the leading cause of death in patients with CKD and increases the progression of CKD.¹⁻³

Chronic disease anaemia (CDA), iron deficiency anaemia (IDA), functional iron deficiency, respectively or simultaneously cause inhibition of erythropoiesis (IRE) and cause anaemia in CKD. This condition adds to the complexity of the diagnosis and management of anaemia in CKD.⁴

Hepcidin, a peptide that has been known as an acute-phase protein, plays a role in the regulation of iron metabolism. Hepcidin helps explain the association of immune response, iron homeostasis, and anaemia of chronic disease. Hepcidin is known to correlate with ferritin, a parameter that is commonly examined for anaemia. Hepcidin has an advantage over ferritin because it directly reflects the status of iron homeostasis, iron availability and the need for erythropoiesis. Hepcidin can be a marker of iron status and a parameter of response to anaemia management in CKD.⁴⁻⁹

In addition to its role in the pathophysiology of anaemia in CKD, hepcidin is currently being studied a lot because it is thought to have a role in the pathophysiology of bone mineral disorders in CKD. Current studies have shown that vitamin D concentration [assessed with serum 25-hydroxyvitamin D (25 (OH) D)] is inversely related to hepcidin concentration and positively associated with haemoglobin and iron concentrations. Recent reports also support a link between vitamin D deficiency and anaemia in patients with CKD, but the role of vitamin D in mineral and bone regulation is not fully elucidated.¹⁰

Vitamin D deficiency is one of the effects of decreased kidney function, with a prevalence rate of up to 80% of all patients with CKD stage 3 or more.¹⁰ Optimal vitamin D status is essential in patients with CKD to regulate parathyroid hormone (PTH) concentrations for bone health, and the prevention of bone mineral disorders known as chronic kidney disease-mineral
bone disorder (CKD-MBD) or translated as bone mineral disorders due to Chronic Kidney Disease.

The risk of anaemia and bone mineral disorders in CKD are two things that need to be considered in the management of CKD patients. Apart from problems in early detection, the treatment of these two conditions also often does not produce the expected results. Much research is devoted to better understanding the pathophysiology of CKD and its complications. The interaction between hepcidin and vitamin D has been widely studied for its role in anaemia and bone mineral disorders in CKD.

**Definition and Classification**

Based on The National Kidney Foundation’s Kidney Disease and Outcome Quality Initiative (NKF-KDQI) in 2002, Chronic Kidney Diseases (CKD) or Chronic Kidney Disease (CKD) is characterized by one of the following criteria. They are kidney damage for at least three months with or without a decrease in glomerular filtration rate (GFR). Abnormalities indicate kidney damage in the structure or function of the kidneys, either manifesting in one of the following signs: blood or urine composition disorders; radiological abnormalities or abnormalities on kidney biopsy. Another criterion was decreased glomerular filtration rate <60 ml/min / 1.73 m² for at least three months, with or without renal impairment. The degrees of CKD is based on the glomerular filtration rate (LFG) as follows: 14 CKD stage 1: LFG 90 ml / minute / 1.73 m² or more; CKD stage 2: LFG 60-89 ml / minute / 1.73 m²; CKD stage 3: LFG 30-59 ml / minute / 1.73 m²; CKD stage 4: LFG 15-29 ml / minute / 1.73 m²; CKD stage 5: FLG <15 ml / minute / 1.73 m² or undergoing dialysis.

**Epidemiology**

The prevalence of anaemia in children with CKD is around 36.6%. The incidence of anaemia increases with the decrease in glomerular filtration rate (GFR), which is around 31-57% at stage 1-2 CKD, 73% in stage 3 CKD, 87% in CKD stage 4 until it reaches 93.3% in CKD stage 5. In the adult population a decrease in haemoglobin (Hb) levels is evident if the LFG decreases <60 mL/minute / 1.73 m. In the pediatric population it has also been reported that an increase in the hazard ratio (HR) for anemia occurs in line with the decrease in LFG, namely at LFG <50.94 mL / minute / 1.73m² (HR 1.954), at LFG <42.84 mL / minute / 1.73m² (HR 2.64), and at LFG <35.5 mL / minute / 1.73m² (HR 3.471).

Research in Ohio on 182 children aged 5-21 years with CKD, reported 50% of CKD stage 2-5 sufferers with vitamin D deficiency. Research conducted by Limori in 2012 showed
that 46 cases of fractures due to osteoporosis in patients with CKD. Research conducted by The Third National Health Assessment and Nutritional Examination reported the incidence of osteoporosis in patients with CKD stage 3-5 as much as 26%.  

Anaemia in chronic kidney disease

CKD anaemia is influenced by many factors, including erythropoietin deficiency (EPO), iron deficiency, inflammation, gastrointestinal bleeding, hemolysis and shortening of erythrocyte age due to uremia also plays a role. Erythropoietin deficiency (EPO) is known to be the leading cause of anaemia in CKD, especially at GFR less than 20 mL/min / 1.73m², so therapy with erythropoietin stimulating agent (ESA) is the primary treatment. Iron deficiency is also a frequent cause of anaemia in CKD, especially in the early stages of the course of the disease, and the symptoms become more pronounced as the LFG decreases. In addition to ESA therapy, iron adequacy must be simultaneously available to meet the needs of erythropoiesis.

CKD anaemia is associated with lower ESA doses and improves with increasing ESA therapeutic doses. Research by Ashby et al. there is a correlation between high hepcidin levels and low ESA therapy. In the administration of ESA therapy, there was a significant decrease in hepcidin levels which lasted 2-4 weeks after the administration of therapy. Researchers also examined levels of growth differentiation factor 15 (GDF15), which functions as a marker of active bone marrow, thereby suppressing hepcidin production. The results of the examination showed that GDF15 levels correlated with ESA dose but not with hepcidin levels, which means that changes in hepcidin levels after ESA administration was not through the GDF15 mechanism.

Erythropoietin resistance means the inability to maintain Hb levels within the expected target by administering the standard EPO dose (> 500 units/kg/week). This situation can be caused by iron depletion, immunologic activity, secondary hyperparathyroidism, chronic blood loss, inadequate dialysis, malnutrition, vitamin deficiency, drugs, aluminium intoxication, and hemoglobinopathy.

Infection and inflammatory factors can inhibit erythropoiesis by suppressing the bone marrow. Although it is not an inflammatory disease, in CKD there are inflammatory factors that play a role so that it can be categorized as anaemia of inflammation (AI) or anaemia in chronic disease (GER). Inflammatory conditions influence resistance to ESA therapy. In this study, there was a correlation between inflammatory markers of CRP and hyporesponsive ESA.
However, in the study of Asby et al., the correlation of hepcidin with inflammatory markers was not proven in multivariate analysis.

**Iron metabolism and erythropoiesis**

Red blood cells or erythrocytes are produced in the bone marrow. Erythrocyte production and maturation is a complex process, involving erythropoietin, hypoxia-inducible factor (HIF), iron, vitamin B12, vitamin C, folic acid, growth hormone, and good milieu. Average red blood cell production in the bone marrow requires adequate iron. However, with the formation of free radicals that cause cell damage due to iron overload, serum iron levels must be controlled. The physiological mechanisms that regulate serum iron levels and iron stores cause humans to retain iron in a soluble form so that it follows the circulation of the blood and goes in and out of cells. The iron balance in the body is regulated primarily by two mechanisms: absorption of iron from the intestines and recycling of iron from senescent erythrocytes. Reticuloendothelial macrophages in the bone marrow and Kupffer cells of the liver and spleen, take erythrocyte senescent and recycle the iron in it (recycle), and store it in the form of ferritin, an iron storage protein. This iron is released back into the circulation via ferroportin channels in the cell membrane based on the need for erythropoiesis.

Hepatocytes play a central role in the regulation of ferroportin. Hepatocytes receive the body's iron status signal and will secrete hepcidin. Hepcidin then interacts with ferroportin to regulate iron release. Hepcidin directly binds to ferroportin and reduces its functional activity due to internalization by iron and then degrades to release the iron into the circulation carried by transferrin.

Transferrin, a transport protein, binds to iron firmly but can release iron molecules and carry them to an active site for erythropoiesis in the bone marrow. When transferrin containing iron encounters the transferrin receptor on the surface of erythroid precursor cells in the bone marrow, iron is released into the cells (Figure 1).
Figure 1. The iron cycle process of senescent erythrocyte into iron Fe in the bone marrow

Hepcidin

Pro-hepcidin, a hepcidin precursor 84-amino acid, is produced by hepatocytes. The hepcidin gene in humans is known as HAMP; OOMIM606464. The next enzymatic process of this molecule produces the bioactive amino acid hepcidin. Hepcidin has a physiological role in regulating iron consumption and regulating iron availability for erythropoiesis and preventing iron overload. Patients with mutations in this gene will experience excess iron, such as hemochromatosis. 7,27,28

Hepcidin is also an acute-phase protein produced by the liver. In diseases with an inflammatory process, high levels of hepcidin play an important role in anaemia due to reticuloendothelial blockade, meaning that the release of iron from reticuloendothelial cells is inhibited so that there is not enough iron needed for erythropoiesis, which means a functional iron deficiency. 26,29,30

Hepcidin regulates blood iron levels by regulating iron absorption from intestinal enterocytes and by inhibiting the release of iron from iron stores (iron recycling) from reticuloendothelial cells.6,26 The mechanism of action of hepcidin is by binding to ferroportin (FPN), a transmembrane found on the cell surface of enterocytes and macrophages and hepatocytes, the primary cells that can release iron into the circulation (Figure 2).

Hepcidin inhibits ferroportin channel function by binding directly to ferroportin channels and triggers internalization and degradation and effectively traps iron in macrophages, hepatocytes, and enterocytes, and prevents the release (export) of iron into the circulation. It prevents iron absorption in the intestine and also prevents the release of iron from the liver and reticuloendothelial system, thereby decreasing the availability of iron carried by transferrin for
erythropoiesis resulting in iron-restricted erythropoiesis. Conversely, low hepcidin levels will cause uncontrolled absorption of iron, resulting in excess iron.6,20,26,27

Figure 2. The mechanism of action of hepcidin by binding directly to ferroportin (FPN), internalization, and degradation of ferroportin 26

Transcription of the HAMP gene is triggered and suppressed by multiple factors; thus, factors that influence hepcidin production are identified. Hepcidin production is triggered by inflammation, iron overload, and iron-replete, on the other hand, hepcidin production is suppressed by erythropoiesis and ESA therapy, as well as in iron deficiency, anaemia and hypoxia (Figure 3).7,20,26,27,31

Hepatocytes respond to an increase in iron saturation from transferrin or an increase in iron stores in the hepatocytes themselves by stimulating hepcidin production by an unknown mechanism. So the physiological response of excess iron via hepcidin is to reduce iron absorption by enterocytes and reduce the release of iron from iron stores in macrophages and hepatocytes.

The synthesis and release of hepcidin are also mediated by bacterial lipopolysaccharides and the release of cytokines, especially interleukin-6 (IL-6). So the hepcidin gene is a gene that is responsive to the acute phase of inflammation. Increased levels of hepcidin triggered by cytokines due to inflammation or infection are considered to be the cause of anaemia due to chronic disease (GER).6,20,24,30,32-34

Inflammation-induced hepcidin is thought to be a self-defence mechanism to limit iron availability during acute bacterial infection. In the case of iron overload, elevated hepcidin is a defence mechanism against high circulating iron. Conversely, suppression of hepcidin production in anaemic or hypoxic conditions aims to increase the amount of iron released from the tissues or iron stores (ferritin), so that it is available for erythropoiesis in the bone marrow.
Erythropoiesis activity itself also suppresses transcription of the HAMP gene and hepcidin levels. 25,26

Increased levels of hepcidin inhibit iron absorption from the gut and select iron in the reticuloendothelial system. Macrophages ingest old red blood cells and, therefore, play a central role in iron recycling. Hepcidin retains iron in macrophages by binding to its receptor ferroportin, the only exporter of iron, causing internalization and degradation, thereby preventing the passage of iron from macrophages into circulation. In inflammatory conditions such as CKD, the antimicrobial peptide hepcidin (called hepcidin) is elevated. Two cytokines (IL-1b and IL-6) generally increase in CKD and stimulate hepcidin production from the liver and macrophages.10

Hepcidin levels increase with decreasing glomerular filtration rate (GFR).20,35 Hepcidin levels can be lowered by intensive dialysis and blocking the inflammatory pathway.24 The immediate goal of reducing hepcidin may be an option for clinicians in the management of anaemia in CKD.

Figure 3. Hepcidin regulation7

Increased levels of hepcidin decrease ferroportin (FPN) expression on the duodenal and reticuloendothelial cell surfaces were leading to reduced intestinal iron absorption, released of iron from storage, and transferrin (TrFn) saturation. In contrast, stimulation of erythropoiesis, stabilization of hypoxia, and iron deficiency suppress hepcidin production, leading to increased intestinal iron absorption, the release of iron from storage, and transferrin saturation so that iron is used more efficiently for erythropoiesis.
Laboratory parameters for the diagnosis of anaemia due to CKD

**Assess the adequacy of iron.**

Assessing the adequacy of iron for erythropoiesis in patients with CKD is not easy. First, it must be known that there are sufficient iron reserves. Then it must be assessed the availability of these iron reserves to reach the bone marrow to form mature erythrocytes. In practical clinical terms, serum ferritin levels are used as a marker of total iron reserves in the body and transferrin saturation as a marker of the availability of iron transport to the bone marrow. Under normal circumstances, about 33% of transferrin is saturated by iron, but this may decrease if the iron supply to plasma from intestinal absorption and iron stores is also reduced. 26,36

Ferritin is an assessment of the status of iron reserves in the body. Serum ferritin levels are challenging to interpret independently as a marker of iron status in inflammation because ferritin is also an acute-phase reactant that increases production in inflammatory conditions. Transferrin saturation measurements are used to determine circulating iron availability for erythropoiesis. Like ferritin, transferrin saturation is also affected by the acute phase of inflammation. In inflammation, the levels of transferrin increase so that the transferrin saturation decreases. 20,36

In healthy children, absolute iron deficiency is considered if serum ferritin is <10 mg / mL, and transferrin saturation is <10%. In children with CKD / ESRD who are on ESA therapy, a higher limit is set, which is said to have iron sufficiency if the serum ferritin level is> 100 ng / mL and the transferrin saturation is> 20%. Although in reality, we often find patients with CKD higher than this value; clinically, the child still has persistent anaemia. Anaemic conditions with serum ferritin levels within normal limits are functional iron deficiency. In this situation, even though there is sufficient iron in the body, the availability of iron for erythropoiesis is not sufficient, and what occurs is iron-restricted anemia.36,37

**Hepcidin is a laboratory marker of iron regulation.**

How to interpret elevated ferritin levels in CKD remains a challenge, whereas the most widely used markers of iron status, ferritin and transferrin saturation, cannot accurately predict response to ESA or iron therapy. The results of the examination are also influenced by inflammation, making interpretation difficult.

In clinical practice, an increase in ferritin levels in CKD often leads to no iron supplementation, because it is feared that it will cause iron overload. People with CKD / ESRD who receive long-term iron supplementation can experience iron overload and buildup. Excess iron can cause side effects such as impaired liver function, heart disease and skin changes, as
well as intravenous iron administration, can cause oxidative stress in patients with CKD. Increasing the iron dose, as well as increasing the ESA dose, in a patient who is hypo-responsive to ESA should take into account the side effects, especially at high doses.

In these patients, if the hepcidin level is checked, and it is known that there is an increase in the hepcidin level, the increase in ferritin does not reflect iron availability for erythropoiesis. However, it is a marker of functional iron deficiency due to the reticuloendothelial blockade. In such circumstances, intravenous iron can be given, because high hepcidin levels will inhibit the absorption of iron given orally so that oral iron supplementation is ineffective. 6,7,26,38

Hepcidin has a positive correlation with ferritin. 39,40 In the event of infection, there is acute hypoferremia associated with both hepcidin-dependent and hepcidin-independent mechanisms. Restrictive hepcidin-dependent hypoferremia due to inflammatory conditions was supported by increasing the proinflammatory marker of interleukin-6 in this study.

Uehato et al. reported a negative association between hepcidin and Hb levels in a group of CKD patients on non-dialysis with high ferritin (> 91 ng / mL). These results indicate that even though the ferritin level is high (meaning that there is a sufficiently high iron reserve), it does not mean that there is enough iron for erythropoiesis in the bone marrow. This is known as iron-restricted anaemia. In the CKD group with low ferritin (<91 ng / mL) there was no evidence of an association between hepcidin and Hb, probably because the mean hepcidin level was also low in this group (7 ng / mL) which indicates absolute iron deficiency. 41

Several studies evaluating hepcidin can predict the response to administration of ESA therapy or iron therapy. Research by Kato et al. found that pretreatment hepcidin assay appears insufficient to predict response to ESA or iron therapy. However, because hepcidin is rapidly regulated, changes in hepcidin levels after intravenous iron administration or ESA may be a better parameter for predicting long-term response. 26

Tessitore et al. examined serum hepcidin levels in 56 HD patients receiving ESA maintenance therapy, to see if it could predict the response to Hb increase with intravenous iron infusion compared with the commonly used marker. The result was that hepcidin levels decreased after intravenous iron administration, but it was not statistically significant. These results suggest that although there is a correlation between hepcidin and ferritin, its usefulness as a predictor of response to ESA is limited to date. Further studies are needed to determine the diagnostic benefits, especially in children. 38

Hepcidin levels in children were studied by Zaritsky et al. Who conducted studies on hepcidin levels in adults and children with CKD compared with healthy controls. The highest hepcidin levels were in children on dialysis (median 652.4 ng / mL), then adults with CKD 2-
4 (median 269.9 ng / mL) and children with CKD 2-4 (median 72.9 ng / mL). In multivariate analysis, hepcidin has a positive correlation with ferritin and hs-CRP. A positive correlation between hepcidin and ferritin has been found in CKD adults, children, and healthy controls.

Another study by Atkinson et al. of 133 children with CKD (LFG 30-90 mL/min / 1.73m²), reported that the lower the GFR, the higher the hepcidin levels and the lower the Hb levels. These results support the role of hepcidin in the development of anaemia in CKD as LFG decreases, and that clinical interventions targeting the hepcidin-ferroportin axis may be independent of erythropoietin.

**Hepcidin and Vitamin D interactions**

Many studies have reported that vitamin D is a regulator of hepcidin. Thus vitamin D also plays a role in the pathophysiology of anaemia, especially anaemia of chronic disease or anaemia in inflammation. Studies in adult populations have shown that supplementation with high doses of vitamin D significantly reduces hepcidin levels.

Moran-Lev reported that children with infections accompanied by anaemia had elevated hepcidin, as well as vitamin D deficiency. Vitamin D levels were significantly lower, and hepcidin levels were significantly higher in patients with anaemic infection than in children with infections without anaemia and control without infection. Subjects with vitamin D deficiency had six times the risk of developing anaemia than those without vitamin D deficiency.

In patients with Chronic Kidney Disease (CKD), it has also been reported that vitamin D insufficiency is significantly associated with anaemia. Studies in CKD patients have shown that vitamin D levels are inversely correlated with the incidence of anaemia and erythropoietin resistance, and are directly related to Hb levels. Hb levels were positively correlated with increased vitamin D levels in patients on peritoneal dialysis.

Vitamin D is known to trigger antibacterial proteins such as cathelicidin (encoded by the cathelicidin [CAMP] gene). Hepcidin was also initially recognized as an antimicrobial protein (encoded by the gene for hepcidin antibacterial protein [HAMP]), which was later recognized as a regulator of iron metabolism. As with cathelicidin, Bacheeta suspects that there is also a role for vitamin D in hepcidin. In vitro research showed that vitamin D therapy decreased mRNA expression for the HAMP gene (which encodes hepcidin) in human monocytes and hepatocytes. Suppression of HAMP expression by vitamin D appears to be direct inhibition of HAMP transcription. Vitamin D therapy also decreases mRNA expression
for ferritin in monocytes and hepatocytes. Immunohistochemical analysis also showed that vitamin D therapy decreased ferritin protein expression. 43

To assess the effect in vivo, Bacheeta also conducted a study on healthy adults by providing vitamin D2 supplementation (ergocalciferol, 1000,000 IU), then checking vitamin D levels before and after supplementation. The results showed that there was an increase in 25D levels, and there was a decrease in circulating hepcidin levels by 34% from baseline at 24 hours after supplementation, and 33% at 72 hours after supplementation. Likewise, there was a significant decrease in ferritin levels between before and after vitamin D supplementation. Another result was a significant increase in levels of fibroblast growth factor 23 (FGF23) and a tendency to decrease in PTH levels. 43

Based on the association of vitamin D deficiency with anaemia that has been reported from various studies, several mechanisms were identified that could explain this association. First, vitamin D has a direct and indirect effect on the action of hepcidin. Second, that vitamin D interacts directly with the hepcidin gene promoter in monocytes and hepatocytes and suppresses hepcidin mRNA transcription. Hepatocyte or monocyte culture therapy with 25-OHD3 or 1,25 (OH) 2D3 showed a halving of hepcidin mRNA expression. 43,45 Third, vitamin D also has an indirect effect on the action of hepcidin by suppressing pro-inflammatory cytokines that stimulate hepcidin production. In inflammatory conditions. 42

CONCLUSION

Hepcidin, as a regulator of iron metabolism, is also influenced by the status of vitamin D in the body. CKD problems and their complications, in particular anaemia and vitamin D deficiency, have interactions that need to be considered in their management.

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