

ROLE OF HEPCIDIN IN MECHANISM OF ANEMIA CHRONIC DISEASE PATIENTS

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Background: Anemia chronic disease (ACD) is an anemia found in certain chronic disease states, typically marked by the disturbance of iron homeostasis or hypoferrremia. This condition leads to shortage of iron for hemoglobin synthesis but the iron storage in bone marrow is left undisturbed. The discovery of hepcidin and its role in iron metabolism has given new insights in anemia chronic disease management. Consecutive sampling method was applied to choose ACD patients at Sanglah General Hospital, Bali-Indonesia. Questionnaire was constructed to note demographic aspect and disease or clinical condition underlies ACD (inflammation, infection, malignancy and others). Hepcidin, Serum IL-6 and CRP level were measured. Sample size and Path analysis mediation method were used to define hepcidin's role on mechanism how anemia develop in ACD patients in which the direct and indirect effects of IL-6 and CRP to hemoglobin (Hb) were counted partially or combined through hepcidin mediation variable. The cumulative influence of IL-6, CRP and hepcidin on anemia (Hb) was only 0.12 or about 12% of hemoglobin level was influenced by IL-6, CRP and hepcidin together whereas the other 93% was influenced by another unknown and unclear factors. Hepcidin could be used as a mediation variable for the development of anemia because the direct influence of IL-6 as exogenous factor was less than its indirect influence through hepcidin. It was not proven for CRP as exogenous variable because the direct influence of CRP to hemoglobin was stronger than the influence of CRP through hepcidin.

Keywords: Hepcidin, IL-6, CRP and ACD

INTRODUCTION

Anemia chronic disease is anemia found in certain chronic disease states, is typically marked by the disturbance of iron homeostasis or hypoferrremia. This condition leads to shortage of iron for hemoglobin synthesis but the iron storage in bone marrow is left undisturbed. Anemia of chronic disease is the second most prevalent anemia after iron deficiency anemia.¹ It can be triggered by wide range of inflammatory disorders such as infection, autoimmune disease, chronic disease, aging process and malignancy, also known as anemia of inflammation.^{1,2} Its morphology is usually normochromic normocytic but it can also be hypochromic microcytic as the disease progresses.² There are some mechanisms related to its pathogenesis, that includes; shortening of erythrocyte's life span, disturbance in erythropoietin production, decreasing of bone marrow response to

erythropoietin and disturbance of iron homeostasis.³

The best management for anemia chronic disease is treatment for its underlying disease. Unfortunately, it is difficult to do because of difficulty in treating the source of infection or difficulty in diagnosing its underlying disease. Furthermore, anemia chronic disease can worsen the prognosis of its underlying disease. Lots of researches are still needed in order to create a new breakthrough in anemia chronic disease management.

Studies of hepcidin, an antimicrobial peptide that have a role as modulator of iron homeostasis has given a new insight for the management of anemia chronic disease.³ Hepcidin, a peptide composed of 25 amino acids, is synthesized by hepatocyte. Data from mouse experiment showed that hepcidin has a role as a negative regulator in small intestine's iron absorption, iron transport in placenta, and iron release from macrophage. Hepcidin's production multiplied up to 100 x in anemia of chronic disease, the fact that showed us hepcidin is the main mediator in anemia of chronic disease. The discovery of hepcidin and its role in

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iron metabolism has given a new insight in the management of anemia of chronic disease.^{4,5}

During infection and inflammation, hepcidin production and concentration were steadily increased.⁵⁻⁷ Many cytokines stimulate hepcidin transcription during inflammation, mainly IL-6 and family of BMP. Studies with mouse and human models showed IL-6 effect in increasing hepcidin production.⁶⁻⁸

CRP is one of the acute phase reactant increased during inflammatory process induced by plasma IL-6 which is produced by macrophages and adipocytes.⁹ All inflammatory processes stimulate the development of IL-1, IL-6, TNF and then stimulate hepatocytes to produce CRP.¹⁰ Increase of CRP can be found during bacterial infection, inflammatory disorders (such as diabetes mellitus, asthma, stroke, and renal failure with regular hemodialysis), smoking, obesity, trauma, myocard infarct, surgery and malignancy.¹¹

Hemoglobin level was associated with the concentrations of some chronic inflammatory markers. The lowest hemoglobin level was found with the highest concentration of inflammatory markers such as IL-6, IL-1, TNF- α and CRP. But statistical analysis concluded that only IL-6 was the independent factor to define hemoglobin level.¹² IL-6 level was inversely related with hemoglobin level in systemic lupus erythematosus patient.¹³ CRP produced by liver as a response of IL-6 was related with mortality.¹⁴ In Rotterdam study, a cohort research of 184 patients, increasing level of CRP was found in 28% breast cancer patients.¹⁵ Meanwhile, another research in breast cancer patients found that there were no differences in CRP level between cancer and non cancer patients.¹⁶

Various inflammatory cytokines in many different diseases associated to anemia chronic disease has played their role in pathophysiology of anemia such as decreasing of erythrocyte's survival, decreasing of iron availability, direct inhibition to hemopoietic stem cell, and inadequate response of erythropoietin to anemia. Increasing of serum cytokines level, especially IL-1, IL-6, IL-10, TNF, IFN- α , IFN- β and CRP have been found in many inflammatory disorders such as AIDS, rheumatoid arthritis, solid malignancy and hematologic malignancy. The role of hepcidin on iron metabolism in those conditions may also play apart. Therefore, this study aims to discover hepcidin role in mechanism of how anemia develop in patient with anemia chronic disease (ACD) at Sanglah General Hospital, Bali-Indonesia.

MATERIALS AND METHOD

Sample was recruited consecutively to choose ACD patients. ACD diagnose was based on defined as anemia with hemoglobin level < 13 g/dL for

male or < 12 g/dL for female and morphologically hypochromic-microcyter (MCV < 80 fl and MCH < 27 pg) and serum ferritin level ≥ 100 μ g/dL occur in certain clinical conditions such as chronic infection, chronic inflammation including autoimmune disease and malignancy, except anemia in chronic kidney disease, chronic liver disease and hypothyroid.¹⁷ ACD patients age above 12 years with PaO₂ ≥ 60 mmHg, normal blood pressure and pulse rate were included. Questionnaire was used to note demographic aspect from samples, disease or clinical condition underlies ACD (inflammation, infection, malignancy and others). Hepcidin level was measured with DRG hepcidin instrument and ELISA method. Serum IL-6 level measured with immune assay solid phase method using ng/ml as measurement unit, whereas CRP level was measured on plasma with normal level less than 3 mg/L.¹⁸ Data collected consecutively through cross-sectional method with role of thumb formula and correction factor 0.35 to fulfill sample size on ACD patients who came to Sanglah General Hospital Bali-Indonesia until the number of samples needed has been achieved. Path analysis mediation method was used to define hepcidin's role on mechanism how anemia develop in ACD patients in which the direct and indirect effects of IL-6 and CRP to hemoglobin (Hb) were counted partially or combined through hepcidin mediation variable. Significance level accepted when $p < 0.05$.

RESULTS

There were 85 ACD patients in this study consisted of 48 male patients (56.47%) and 37 female patients (43.5%) with underlying disorders as listed in Table 1.

Table 1
Characteristic of Subject

Characteristic	Amount
Sex, n (%)	
male	48 (56.47%)
female	37 (43.52%)
Diagnose	
Chronic infection	25 (29.41%)
Inflammation	6 (7.05%)
Malignancy	31 (36.47%)
Others	23 (27.05%)
Age (mean $\pm$ SD)	43.26 $\pm$ 16.42
Hb (mean $\pm$ SD)	7.58 $\pm$ 2.03
HCt (mean $\pm$ SD)	24.58 $\pm$ 5.80
MCV (mean $\pm$ SD)	72.71 $\pm$ 8.48
MCH (mean $\pm$ SD)	21.91 $\pm$ 4.44
WBC (mean $\pm$ SD)	13.74 $\pm$ 22.86
Platelet (mean $\pm$ SD)	353.47 $\pm$ 175.59
BUN (mean $\pm$ SD)	14.36 $\pm$ 8.51

Table 1 continued

Characteristic	Amount
Creatinin (mean±SD)	0.73± 0.26
SGOT (mean±SD)	37.29±22.47
SGPT (mean±SD)	30.59± 24.82
Serum_iron (mean±SD)	31.03±39.12
TIBC (mean±SD)	229.39±116.27
Sat transferrin (mean±SD)	17.50±21.50
Ferritin (mean±SD)	686.23±759.57
ESR-1 (mean±SD)	11.93±14.78
ESR-2 (mean±SD)	61.32±41.62
CRP (mean±SD)	62.89±75.12
Hepcidin (mean±SD)	53.62±48.27
IL-6 (mean±SD)	74.73±94.91

Correlation matrix showed a significant correlation between hepcidin variable with hemoglobin, TIBC, transferrin saturation, ferritin, erythrocyte sedimentation rate (ESR), CRP and IL-6. Correlation between hepcidin and IL-6 was 0.641 with $p = 0.000$. This correlation was quite strong in the same direction, which meant the higher IL-6 level would be followed by higher hepcidin level. It was the same with correlation between IL-6 and CRP which was found with $r = 0.519$ and $p = 0.000$. Although this study failed to show the correlation between IL-6 and hemoglobin level, it found a correlation between hepcidin and hemoglobin with $r = 0.297$ and $p = 0.006$.

This study wanted to know how much the effect of some variables such as IL-6, CRP and hepcidin causing anemia both directly and indirectly through hepcidin. We chose path analysis mediation model with hepcidin as mediation variable. Path model chosen was shown below.

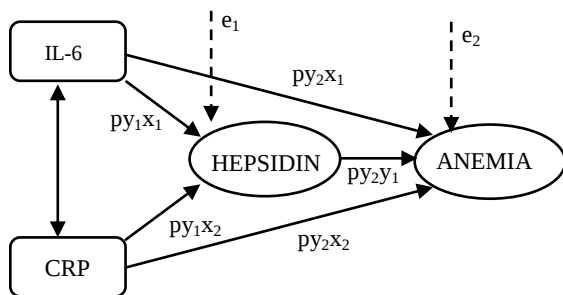


Figure 1

Path model to discover the role of hepcidin as mediation variable for IL-6 and CRP's effect in causing anemia

Structural equation with first and second substructures was:

$$Y_1 = pY_1X_1 + pY_1X_2 + e_1$$

$$Y_2 = pY_2X_1 + pY_2X_2 + PY_2Y_1 + e_2$$

Path analysis of the first substructure, partial/direct influence of IL-6 variable on hepcidin was 0.64 known as beta weight with $p = 0.0001$. However, partial/direct influence of CRP on hepcidin was only 0.01 with $p = 0.945$. The cumulative influence of IL-6 and CRP on hepcidin was indicated by $R^2 = 0.41$. It meant that cumulative influence of IL-6 and CRP variable on hepcidin was only 41% and the remainder ($e_1 = \sqrt{1 - R^2}$) approximately 64% was explained by another factor.

Feasibility of regression model for the first substructure as indicates by ANOVA test's which was significance with $p = 0.0001$. IL-6 and CRP as predictors (exogenous variables) chosen in this model was right because standard error of estimate of endogenous variable (hepcidin) < standard deviation (37.51<48.27). The existence of autocorrelation if $DW < 1$ or > 3 can be seen on Dubin-Watson (DW) numeric in model summary table. DW score in this study was 1.75. Multi-collinearity occurs if there was a very strong correlation or approaching 1 between exogenous variables. However, correlation between IL-6 with CRP in this study was only 0.52. Normality and linearity of data in this model have been fulfilled because there were bell curve shape and normal plot regression showing data scatter around the straight line. The feasibility of regression coefficient (beta weight) of exogenous variables showed that only IL-6 had beta weight 0.64 and statistically significant with $p = 0.0001$, whereas CRP had beta weight 0.01 with $p = 0.945$. Because all requirements had been fulfilled, this first regression model (first substructure) in this path analysis mediation model was right and feasible. Based on this, structural model for the first substructure was arranged as below:

$$\text{Hepcidin} = 0.61 \text{ IL-6} + 0.01 \text{ CRP} + 0.64$$

Based on path analysis of the second substructure, partial/direct influence of IL-6 variable on anemia (Hb) was only -0.12 (beta weight) with $p = 0.437$. On the other hand, partial/direct influence of CRP on anemia (Hb) was 0.1 with $p = 0.092$. Cumulative influence of IL-6, CRP and hepcidin on anemia (Hb) was only $R^2 = 0.12$. In short word, cumulative influence of IL-6, CRP and hepcidin variables on anemia (Hb) was only 12% and the remainder 93% ($e_2 = \sqrt{1 - R^2}$) was explained by another factor.

The feasibility of regression model for the second substructure can be seen on ANOVA test of significance with $p = 0.015$. So this regression model was right and feasible. Predictors (exogenous variables) chosen in this model such as IL-6, CRP and hepcidin was quite right because standard error of estimate of endogenous variable (anemia) < standard deviation with $1.93 < 2.03$.

Autocorrelation if $DW < 1$ or > 3 . DW score in this study was 2.29. Multi-collinearity happened if between exogenous variables, there was a very strong correlation or approaching 1, whereas none of the correlation between IL-6, CRP and hepcidin in this study was approaching 1. Normality and linearity of data in this model had also been fulfilled, because there was bell curve shape on data distribution and straight line data scatter on normal plot regression. The feasibility of regression coefficient (beta weight) of all exogenous variables showed that only hepcidin has beta weight 0.30 and statistically significant with $p = 0.03$ whereas CRP and IL-6 had beta weight 0.21 and -0.12 with $p > 0.05$. Because all requirements were fulfilled, this second regression model (second substructure) in this path analysis mediation model was right and feasible, and structural model for this second substructure could be arranged as below

$$\text{Anemia (Hb)} = -0.12 \text{ IL-6} + 0.21 \text{ CRP} + 0.30 \text{ hepcidin} + 0.93$$

From this study, path analysis model from exogenous variables of IL-6 and CRP on anemia (Hb) through hepcidin variable could be drawn as below.

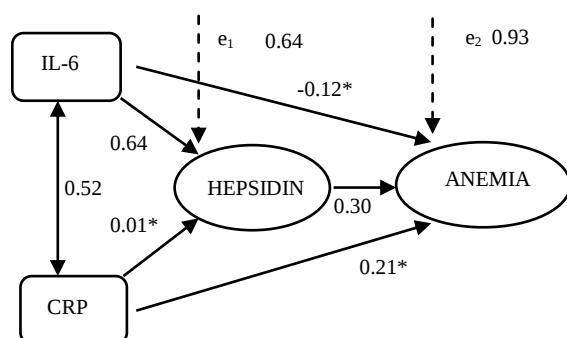


Figure 2

Path diagram of analysis result showing the influence of IL-6 and CRP variable to anemia (Hb) directly and indirectly through hepcidin variable

DISCUSSION

This study found that IL-6 could influence the development of anemia through hepcidin although the rate seems dismal. Contrary to IL-6, CRP has no influence on hemoglobin on ACD patients either directly or indirectly. This could happen because CRP as well as hepcidin is an acute phase protein that isn't produced or secreted in the same time and same concentration in certain inflammation reactions, although both of them were secreted by hepatocytes through IL-6 and another cytokines such as IL-1, TNF- α and IFN- γ .¹⁹ Person analysis found that significant correlation between CRP with hepcidin, but CRP was not influence hepcidin

and hemoglobin level ($p > 0.05$). Although the increment of different acute phase proteins occurs together, they were not increase with the same proportion in the same time. The discrepancy of this increment was frequently found even some of them was not increase at all. This could be explained because of the differences in cytokine production by different diseases.²⁰ For example, the differences of secretion level of CRP and ESR in systemic lupus erythematosus/SLE patients in which LED increased but CRP level was still in normal level.²¹ There were many clinical conditions did so, therefore none of the single examination could definitely proved the existence of inflammation processes. Besides that, a wide variation of underlying diseases causing ACD could cause different cytokine production.

CRP was the most stable inflammatory marker. Increasing level of CRP was stimulated by IL-6 that produced by macrophages and adipocytes. All inflammation processes could stimulated the development of IL-1, IL-6, TNF which in turn stimulated hepatocytes to produce CRP.^{9,10} High level of CRP can be seen during bacterial infection, inflammatory disorders (diabetes mellitus, asthma, stroke, and renal failure with regular hemodialysis), smoking, obesity, trauma, myocard infarct, surgery and malignancy.¹¹ The important role of CRP in body defense mechanism is to remove necrotic cell and other abnormal cell through inflammation and anti inflammation processes involving cytokines and tissue factor. Nonetheless the main effect of CRP was its anti inflammatory property. This could be seen on animal study when transgenic mouse in which CRP expression decreased the degree of inflammation process induced in lung and joint tissue. Accumulation of neutrophils was found less in inflammation site. Based on animal study, there was no direct effect and influence of CRP on anemia in inflammatory disorders (ACD), as also can be seen that CRP did not significant influence the development of anemia in this study.

Anemia chronic disease, anemia that found in certain chronic disease states, is typically marked by the disturbance of iron homeostasis or hypoferremia. This condition leads to shortage of iron for hemoglobin synthesis but the iron storage in bone marrow is left undisturbed. It can be triggered by wide range of inflammatory disorders, either acute or chronic, such as infection (bacterial, viral, fungal or parasite infection), autoimmune disease, and malignancy, so it is also known as anemia of inflammation.^{1,2} But it also happened in non inflammatory conditions such as geriatric conditions and diseases related with aging process like congestive heart disease. Inflammation reaction in aging process was marked by a mild increase of inflammatory markers such as IL-6 and CRP. It was found higher in geriatric patients with

anemia than geriatric patients without anemia.^{2,3} In acute inflammation condition, such as critical ill condition in intensive care unit, this anemia can be acutely happened (in some days). It's caused by the inflammation condition itself then worsen by blood loss or erythrocyte destruction as a result of its underlying disease or iatrogenic process.⁴ Because there are many clinical conditions presenting anemia similar to the anemia of ACD, therefore many different study frequently come up with inconsistent results as well as the recent study showed no clear influence of CRP on hepcidin and anemia. Study in Rennes, France, reported that there was no correlation between hepcidin expression for CRP as an inflammatory indicator in patient underwent liver operation caused by malignancy. It is thought because of the heterogeneity of population samples, the possibility of less sensitive CRP in describing inflammation and the role of disturbing factors that couldn't be removed.²² The same result was also found in hemodialysis patients in which there was no correlation found between coefficient variation of CRP with coefficient variation of hepcidin ($p = 0.44$).²³ Kato et al. also wasn't found any significant correlation between CRP and hepcidin in hemodialysis patients and supposed that it was caused by differences in the half live of CRP and hepcidin.²⁴ Study on peritoneal dialysis patients in Japan found the insignificant correlation between CRP level with hepcidin serum ($p = 0.722$). It was noted that the level of inflammatory cytokines increased not too high, so supposedly that an inflammation processes with certain degree was needed to induce hepcidin production.²⁵ This was supported by Koury's report that stated temporary role of hepcidin in which hepcidin level increased higher in early of inflammation process then decreased in chronic process.²⁶

The newest study showed that hepcidin worked on iron homeostasis by binding to ferroportin then followed with internalization and degradation of ferroportin in lysosome. The removal of ferroportin from cell membrane decrease the export of intracellular iron to plasma.²⁷ In normal or increasing iron stores, liver will produce hepcidin that circulates to duodenum. Then hepcidin bound to ferroportin, followed by ferroportin's internalization so iron transport from duodenal enterocytes to plasma would be inhibited. On the other hand, when iron stores decrease, hepcidin production decreased, ferroportin molecule will be expressed in basolateral membrane of enterocytes to increase iron transport from enterocytes to plasma transferrin. Hepcidin-ferroportin interaction is also found in macrophages, in which high hepcidin concentration will be followed by the binding of hepcidin to ferroportin and ferroportin internalisation. It will

inhibit iron export to plasma and caused iron retention on macrophages especially macrophages in lien.²⁷ However, a next study is still needed to define the cut-off of hepcidin level required for ferroportin degradation.²⁸ The simple mechanisms of action of hepcidin are limiting iron absorption in duodenum, iron recycling in macrophages, iron release from liver iron stores and iron transport in placenta.^{5,6} Experiments in mouse injected with hepcidin found a dramatic decrease of serum iron level (75%) in 1 hour and hepcidin effect could last for 72 hours. This condition possibly caused by the time required to re-synthesis hepcidin receptor (ferroportin) in sufficient quantity.²⁹

This study found significant influence of IL-6 on hepcidin with beta weight of 64%. The important role of IL-6 in this hepcidin pathway was supported by many study results either on animal or human. In one study, turpentine injection increased hepcidin level 6 times and decrease serum iron level 2 times. This effect disappeared when it is done on mouse without hepcidin gene.³⁰ In another animal, hepcidin injection caused hypoferremia in 4 hour and got worsed when it is implanted by hepcidin-excreting tissues.¹⁸ In experimental human suffered from falciparum malaria, the treatment given would induce the increasing of IL-6 and hepcidin serum at the same time as the condition of low serum iron level, high ferritin serum level, and low hemoglobin level in young reticulocytes.³¹ Another evidence showed no effect of turpentine injection on increament of hepcidin expression when done on animal with hepcidin gene knocked-out. The increasing level of hepcidin in hepatocyte culture after bacterial lipopolysaccharide injection would disappear when this LPS injection combined with IL-6 antibody. IL-6 infusion to volunteer immediately followed by increasing level of hepcidin expression on urine and decreased serum iron level.⁶

Another human study showed increased of urinary hepcidin excretion 7.5 times in 2 hours after IL-6 infusion and then followed by decreasing of serum iron level and transferrin saturation by 30%. It showed that IL-6-hepcidin axis played an important role in hypoferremia occurs in inflammation.^{4,5}

This study showed hepcidin influence on hemoglobin level was 30% (partial influence of hemoglobin level in this case), whereas cumulative influence of IL-6, CRP and hepcidin on hemoglobin was 12% and cumulative influence of IL-6 and CRP on hepcidin was 41%. Meanwhile direct influence of IL-6 on hemoglobin was 12% and IL-6 influence on hemoglobin through hepcidin was 19% (seen in figure 2). Although there's only a small differences, but hepcidin role as a mediator variable can be seen. As known, regulation of hepcidin expression isn't only by IL-6 but there are

still many factors modulating hepcidin level. Hepcidin regulation is also influenced by body iron status either serum iron or intracellular iron. Another factors such as anemia, hypoxia, and erythropoietic activity could also influence the regulation of hepcidin production. Hepcidin synthesis in mouse increased in 1 day after high iron meal and urinary hepcidin concentration increased in less than 1 day after high iron meal.^{4,5} Besides that, study by Nemeth et al. in Los Angeles, 2003, showed that iron load given to hepatocytes would decreased 50% hepcidin mRNA. The higher iron load given the higher the decreased hepcidin mRNA.⁶ A fact that the defect of HFE, Tfr and hemojuvelin decrease hepcidin level, would raise a hypothesis that many different forms of hemochromatosis is caused by iron inability to stimulate hepcidin synthesis.³² These raised a question whether iron played its role on hepcidin synthesis.^{4,5} This hypothesis supported by study on animal where on two different type of anemia that developed either because of bleeding after repeated phlebotomy or hemolytic anemia after fenilhydrazine given, showed that iron level in liver was unchanged. It thought that the negative influence of anemia on hepcidin gene expression was more dominant than positive influence of iron and was supposed mainly through hypoxia.³⁰ Study by Park et al., 2006, showed that hepcidin mRNA level correlated positively with serum iron level but wasn't correlated with hemoglobin level. Author concluded that anemia influenced hepcidin expression mainly through indirect way.³³

Hypoxia is also potent inhibitor of hepcidin expression, even without anemia. This decreasing of hepcidin synthesis is supposed to happen through Hypoxia-Inducible Factor (HIF) pathway influencing hepcidin expression.⁶ In normal condition, HIF- α subunit hydroxylated by oxygen. But on hypoxic condition, hydroxylation activity is inhibited rendering HIF- α subunit accumulated, translocated to nucleus, heterodimerisation and bound to HREs (hypoxia-responsive promoter elements) of hepcidin gene lead to hepcidin gene transcription inhibited.³⁴

How erythropoietic activity influencing hepcidin is shown on the study from Ashby et al. in London, 2010. It reported that a significant decrease of plasma hepcidin level started 24 hour after subcutaneous erythropoetin, approaching maximal on the third day, and recover gradually after 2 weeks. The decreasing of hepcidin expression by erythropoetin is suggested caused by increase of bone marrow activity. Supposed 3 mechanisms involve such as the use of iron by bone marrow as a response to erythropoetin, followed by the decreasing of hepcidin synthesis through decreasing of transferrin saturation recognized by hepatocytes. The second mechanism

is involving soluble transferrin receptor, an indicator of circulated bone marrow iron requirement. Study showed that soluble transferrin receptor would increase during erythropoesis and inversely correlate with urinary hepcidin excretion.³³ The third mechanism is supposedly involving two proteins produced by erythroid precursor in bone marrow ie GDF15 (Growth Differentiation Factor15) and TWSG1 (Twisted Gastrulation Protein1). Although mouse study showed that the disturbance of GDF15 isn't accompanied by the disturbance of body iron status, higher dose of GDF15 is needed to inhibit the synthesis of hepcidin mRNA on in vitro study. Besides that, study said that GDF15 suppress hepcidin expression in hepatocytes culture and GDF15 in thalassemia- β patient found in higher level. This may explain how the decreasing of hepcidin expression in excess of body iron stores and support the opinion about GDF15 role as a hepcidin inhibitor mediator.⁶

Hemoglobin level was associated with several different inflammatory marker ie IL-6, IL-1, TNF- α and CRP but statistical analysis concluded that only IL-6 was the independent factor defining hemoglobin level.¹² Other study found that IL-6 level negatively correlated with hemoglobin level in systemic lupus erythematosus patients.¹³ Hemoglobin level in ACD is defined by many factors besides hepcidin role induced by IL-6. Anemia in ACD is a combination of many different factors that interact, and the development of anemia in ACD involved many mechanisms including disturbance in erythropoetin production, decreasing of bone marrow response to erythropoetin and disturbance of iron homeostasis, shortening of erythrocyte's lifespan.³⁵

The shortening of erythrocyte's lifespan during inflammatory processes supposedly caused by increase of erythrophagocytosis and erythrocyte destruction due to cytokines and free radicals.¹ In one study of 70 adult malignancy patients including hematologic malignancy, inverse correlation between the degree of anemia and the level of endogenous erythropoetin was found with immunoassay method examination.³⁶ However the influence of hepcidin negative regulation on development of erythroid precursor in vitro was also found.² The pathogenesis of anemia of chronic disease related with the disturbance of iron homeostasis is still the main problem that need to elucidate. The discovery of hepcidin has given a new advancement to solve this problem.³ There are many different mechanisms occurs with different intensity in every diseases and clinical conditions underlying the development of anemia in chronic disease patients. They are supposed playing their role in contributing the inconsistent result of

studies regarding influence of iron parameter and inflammatory mediator markers.

The weakness of this study was the variability of the main diseases underlying ACD, both in pathogenesis and biomolecular aspects. As a consequences, the role of each variable examined was very inconsistent in times and concentrations. It was supposedly play apart on this study and also this study was unable to recruit sufficient number of patients due to certain conditions.

CONCLUSION

The partial influence of IL-6 on hepcidin was 0.64 or about 64% of hepcidin level was partially influenced by IL-6 level. The partial influence of CRP variable on hepcidin was 0.01 or about 1% of hepcidin level was partially influenced by CRP level.

The cumulative influence of IL-6 and CRP on hepcidin was 0.41 or about 41% of hepcidin level was influenced by IL-6 and CRP together whereas the other 64% was influenced by another unknown and unclear factors.

The partial influence of IL-6 on anemia (Hb) was only -0.12 or about 12% of hemoglobin level was partially influenced by IL-6 level.

The partial influence of CRP on anemia (Hb) was 0.21 or about 21% of hemoglobin level was partially influenced by CRP level.

The partial influence of hepcidin on anemia (Hb) was 0.30 or about 30% of hemoglobin level was partially influenced by hepcidin level.

The cumulative influence of IL-6, CRP and hepcidin on anemia (Hb) was only 0.12 or about 12% of hemoglobin level was influenced by IL-6, CRP and hepcidin together whereas the other 93% was influenced by another unknown and unclear factors.

Hepcidin could be used as a mediation variable for the development of anemia because the direct influence of IL-6 as exogenous factor was less than its indirect influence through hepcidin. It was not proven for CRP as exogenous variable because the direct influence of CRP to hemoglobin was stronger than the influence of CRP through hepcidin.

FUTURE WORK

Knowing that many different conditions underlying ACD could play different role on how to develop anemia in ACD patients, the future research should be done just in one particular disease causing ACD.

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