ABSTRACT AND PROGRAM BOOK

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The Indonesian Society of Haematology and Blood Transfusion
and
Asia-Pacific Blood and Marrow Transplantation Group

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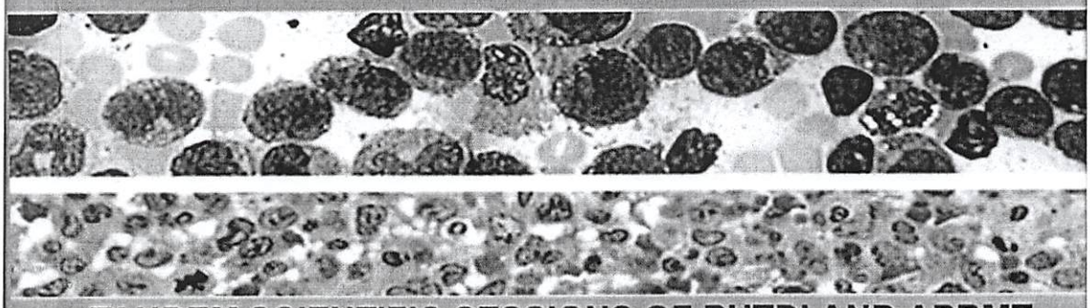
APBMT
Asia Pacific Blood and Marrow Transplantation Group

DOING OUR PART TO SAVE HUMAN LIVES

THE ASIA-PACIFIC COLLOQUIUM ON HAEMATOLOGY



TANDEM SCIENTIFIC SESSIONS OF PHTDI AND APBMT

J.W. MARRIOTT HOTEL - MEDAN

WORKSHOP 4 September 2015
SYMPOSIA 5-6 September 2015

Welcome Message

President, PHTDI (The Indonesian Society of Hematology and Blood Transfusion)

Dear Colleagues

Welcome to Indonesia and to the Asia Pacific Colloquium on Hematology: A Tandem Scientific Meeting of the Indonesian Society of Hematology and Blood Transfusion (INASHBT) / Perhimpunan Hematologi dan Transfusi Darah Indonesia (PHTDI) and the Asia Pacific Bone Marrow Transplantation (APBMT) Group.

It is such an honour for the INASHBT to host this tandem meeting. A tandem or joint meeting is a special scientific meeting in the new concept that two institutions are making a progress towards one unity. In many other parts of the world this joint meeting happens quite often. However, for the INASHBT this is the first time to hold a joint meeting with APBMT Group. In the past time, the INASHBT has had several times to be a host of the international scientific meetings, such as with the International Society of Blood Transfusion (ISBT) , with the Thalassemia International Federation (TIF), with the Asia Pacific Iron Academy (APIA).

This year the Annual Scientific Meeting of the INASHBT on hematology and hemostasis thrombosis will be held in Medan , North Sumatera, in September 2015. In conjunction with this annual meeting, the INSHBT will also collaborate with the APBMT Group to organize a joint meeting on bone marrow transplantation(BMT) , with a hope that the both sides can gain knowledge and experiences reciprocally with regards to hematopoietic stem cell transplantations (HSCT), either in hematological malignancies or in other hematological non malignancy diseases.

There are currently 15 centers of hematology (going to be 17 centers) throughout Indonesia. However, only 3 centers has started with BMT in a period of 1987-1989 which included Semarang, Yogyakarta and Jakarta. Jakarta started conducting autologous and allogeneic BMT in acute leukemias and chronic myeloid leukemias, followed by peripheral blood stem cell transplantation (PBSCT) started in 1997. However, due to monetary crisis in 1998 as well as the old facilities for BMT, there has been a period of no activities of BMT until the renovation of the facilities, the building of hematopoietic stem cell blood banking and processing (cGMP)

MOLECULAR ASPECT CORRELATION BETWEEN C-REACTIVE PROTEIN (CRP) AND CONTENT HEMOGLOBIN RETICULOCYTE(CHR) IN CHRONIC KIDNEY DISEASE (CKD) HEMODIALYSIS PATIENTS

Indranila KS, Hastim N
Departement Clinical Pathology Medical Faculty University Of Diponegoro Semarang

End-stage Chronic Kidney Disease (CKD) undergo hemodialysis, typically had chronic inflammation that occurs due to multifactorial, which lead to functional iron deficiency . Functional iron deficiency in CKD change the imbalance between the need iron for erytropoiesis and iron release from Reticulo endothelial system (RES). C-reactive protein (CRP) is a marker of inflammation occur are still to be determined. Hemoglobin reticulocyte content (CHr) are parameter that describe the availability of iron status on this relationthip, reflect how much iron in the bone marrow for the formation of erythrocyte 3-4 days earlier. The aim of this study is to examine the relation between C-reactive protein and Content Hemoglobin reticulocyte in Chronic Kidney Disease undergo hemodialysis. Observational study with cross sectional approach in 31 patients with CKD undergo hemodialysis, in dr. Kariadi hospital. Laboratory assays performed on blood samples for, CRP, and CHr. CRP was determined quantitatively by immunoturbidimetry, and CHr was checked by flowcytometri. The relationship between variables were analyzed with the Spearman rank test. Median serum CRP was 2,15 mg/dL and CHr median was 29,5 pg. Spearman correlation test results between CRP with CHr was P,0,05. There is a relationship between CRP and CHr in CKD patients undergo hemodialysis.

Molecular aspect Correlation between C-reactive protein (CRP) and Content hemoglobin reticulocyte(CHr) in chronic kidney disease (CKD) hemodialysis patients

Indranila KS, Hastim N

nila_fkundip@yahoo.com

Departement Clinical Pathology Medical Faculty University Of Diponegoro Semarang

Abstract :

Background : End-stage Chronic Kidney Disease (CKD) undergo hemodialysis, typically had chronic inflammation that occurs due to multifactorial, which lead to functional iron deficiency . Functional iron deficiency in CKD change the imbalance between the need iron for erytropoiesis and iron release from Reticulo endothelial system (RES). C-reactive protein (CRP) is a marker of inflammation occur are still to be determined. Hemoglobin reticulocyte content (CHr) are parameter that describe the availability of iron status on this relationthip, reflect how much iron in the bone marrow for the formation of erythrocyte 3-4 days earlier.

Aim of this study : We aimed to examine the relation between C-reactive protein and Content Hemoglobin reticulocyte in Chronic Kidney Disease undergo hemodialysis .

Methods : Observational study with cross sectional approach in 31 patients with CKD undergo hemodialysis, in dr. Kariadi hospital. Laboratory assays performed on blood samples for, CRP, and CHr. CRP was determined quantitatively by immunoturbidimetry, and CHr was checked by flowcytometri. The relationship between variables were analyzed with the Spearman rank test.

Results : Median serum CRP was 2,15 mg/dL and CHr median was 29,5 pg. Spearman correlation test results between CRP with CHr was P,0,05.

Conclusion : there is a relationship between CRP and CHr in CKD patients undergo hemodialysis.

Keywords : CKD, iron deficiency, CRP, CHr, hemodialysis.

1.Introduction

Chronic Kidney Disease (CKD) is a pathological process with diverse etiology, resulting in a progressive decline in renal function, may end up with kidney failure and require renal replacement therapy such as dialysis or transplantation ginjal.1) prevalence of CKD in the world is quite high, in the United States In 2009 as many as 116 395 people.2) In Indonesia in 2009 amounted to 12.5%, or about 18 million people. 3) Data in Hospital Dr. Kariadi, during the period February 2010 - February 2012, CKD patients numbered 43 people and who died in the ICU and HCU number 27 (62.8%). 4) In Indonesia, hemodialysis (HD) done 2 times a week for 4- 5 hours .5) Based KDOQI, HD advised performed 3 times in 1 minggu.6) Tbahriti et al assert that increases with the progression of inflammatory markers CKD.7), and a change in the balance of iron in the body, which is typical of anemia of chronic disease (ACD) or anemia of inflammation.8)

C-reactive protein (CRP) is one of the acute phase protein that increases in response to inflammation, infection, malignancy, or cardiovascular disease. CRP is also a predictor of mortality in patients with end-stage CKD. CRP production in the liver triggered by IL-6.9) Nand et al showed that the average dose of erythropoietin needed to maintain hemoglobin increased by 30-70% in patients on dialysis with serum CRP> 2 mg / l compared to patients with lower CRP. 10)

Reticulocyte hemoglobin content (CHr) is an alternative parameter that describes the availability of iron by measuring the hemoglobin content of reticulocytes. CHr reflects how much iron in the bone marrow for the formation of erythrocytes 3-4 days before the examination .11) CHr much done in patients undergoing dialysis. CHr showed a sensitivity of 100% and specificity of 80% as a predictor to determine response to therapy compared to serum ferritin iron or saturation transferin.12) Miwa CHr et al perform measurements in hemodialysis patients with anemia, it was found that CHr is a sensitive and specific marker for measuring the iron status dialysis patients.**13)**

Hackeng et al say Chr related to the percentage of hypochromic red cells (Hypo%) (rho-0.63 p <0.0001), STfr (rho 0.26 p <0.05), and log CRP (rho-0, 50 p <0.0001) .14) El Khatib et al also conducted studies in hemodialysis patients, it was found that in the group of high CRP (> 5mg / L) obtained more patients who have CHr and low STfr (CHr <29pg and STfr < 20).15)

The purpose of this study prove the existence of a relationship between CRP levels with CHr in patients with end-stage CKD who undergo hemodialysis.

2. Chronic Kidney Disease (CKD)

Based on Kidney Dialysis Outcome Quality Initiative (KDOQI), CKD is a condition of the kidney damage that occurs during the 3 months or more, based on pathologic abnormalities or markers of kidney damage such as abnormalities in the urinalysis examination by a decrease in glomerular filtration rate or tidak.16) Criteria for the diagnosis of CKD according to the KDOQI : 1). ≥ 3 months kidney damage, structural or renal function abnormalities; with or without a decrease in glomerular filtration rate , pathologic abnormalities or markers of kidney damage such as abnormalities in the

composition of blood , urine or abnormalities on radiology examination.2) glomerular filtration rate <60 mL / min / 1,73m2 for> 3 months, with or without renal damage.16)

Pathophysiology of CKD at first depending on the underlying disease, but further processes that occur more or less are same. Future reduction of renal hypertrophy resulting in structural and functional nephrons remaining (surviving nephrons) as a compensation, which is mediated by vasoactive molecules such as cytokines and growth factors. This resulted in hyperfiltration, which was followed by an increase in capillary pressure and glomerular blood flow. This adaptation process was brief, which would be followed by a process maladaptation form nephron sclerosis which still remain.17) This process eventually followed by a progressive reduction in nephron function, although essentially the disease is no longer active. At the earliest stages of chronic kidney disease, there is a progresive loss occurs in nephron function, there is also loss in kidney/renal reserve, in cases where basal glomerular filtration rate (GFR) is normal or incresed.18) Slowly but surely there will be a progressive reduction in nephron function, which is characterized by elevated levels urea and serum creatinine. Until the GFR by 60% of patients still do not feel the complaint (asymptomatic), but has been an increase in urea and creatinine serum levels. After living 30% GFR began to occur in patients with such complaints nocturia, weakness, nausea, lack of appetite and weight loss.19) Until GFR below 30% to 15%, patients showed signs and symptoms , symptoms of uremia real as anemia, increased blood pressure, phosphorus and calcium metabolism disorders, pruritus, nausea, vomiting, and so forth. Patients are also susceptible to infections such as urinary tract infections, respiratory tract infections, and gastrointestinal infections, also will occur the water balance disorders such as hypo- or hypervolemia, electrolyte balance disorders, (sodium and potassium). At GFR below 15% will be symptoms and serious complications, and patients already requiring renal replacement therapy /renal replacement therapy, among others, dialysis or kidney transplantation. In this situation the patient is said to end-stage renal failure. 20) CKD stage classification is determined by the glomerular filtration rate, a higher stage shows the value of the glomerular filtration rate is lower. (Table 1) This classification is important to guide therapy and commencement conservative replacement therapy renal physiology. KDOQI guidelines recommend to calculate GFR in adults with the Cockcroft-Gault formula or formula MDRD.21)

Table 1. Stage in CKD

Stage	Description	GFR(ml/min/1,73m^2)
1	Kidney damage with normal GFR	>90
2	Kidney damage with mild decrease GFR	60-80
3	Kidney damage with decline GFR	30-59
4	Kidney damage with severe decresed GFR	15-29
5	Kidney failure	<15 or dialysis

Inflammation in patients with CKD

Patients with kidney disease have increased proinflammatory cytokines associated with infections and a variety of stimulation. According to available data, the levels of proinflammatory cytokines in hemodialysis patients 8-10 times higher than in healthy humans. 22)

Factors that cause inflammation in patients with renal failure, among others, uremia, dialysis contaminated, bioincompatible membrane dialysis, reduction in cytokine clearance by the kidneys, persistent infection, the accumulation of advanced glycation end products, a decrease in plasma antioxidant capacity, the burden of atherosclerosis, autoimmune diseases , and the transfer of endotoxins through intestinal. .23)
More than 30-65% of patients with end-stage CKD occurs activation of inflammatory responses such as increased levels of CRP serology, αpro-inflammatory cytokines IL-1, IL-6 and TNF-α. According to previous studies, there is a relationship between inflammatory markers indirectly affect **Hb variability** through its role in the inflammatory cytokines existing iron metabolisme .24) inflammatory cytokines can affect the development of anemia through several mechanisms, likely through erythropoiesis bone marrow suppression, suppression of erythropoietin production, gastrointestinal bleeding and modulation iron metabolisme. 25) Hepsidin, hormone which responsible for the body's iron balance affects the metabolism of iron in anemia in CKD. Hepsidin will bind ferroportin (transmembrane proteins) and then degrade these proteins , resulting in decreased export of iron and retention of cellular iron. Hepsidin synthesis increases in response to anemia, hypoxia, and erythropoiesis. Hepsidin expression also increased during infection and inflammation that causes hipoferremia and decreased its supply to the bone marrow. IL-6 is released during inflammatory processes and is associated with increased gene expression hepsidin both in mice and humans. (Figure1) 26)

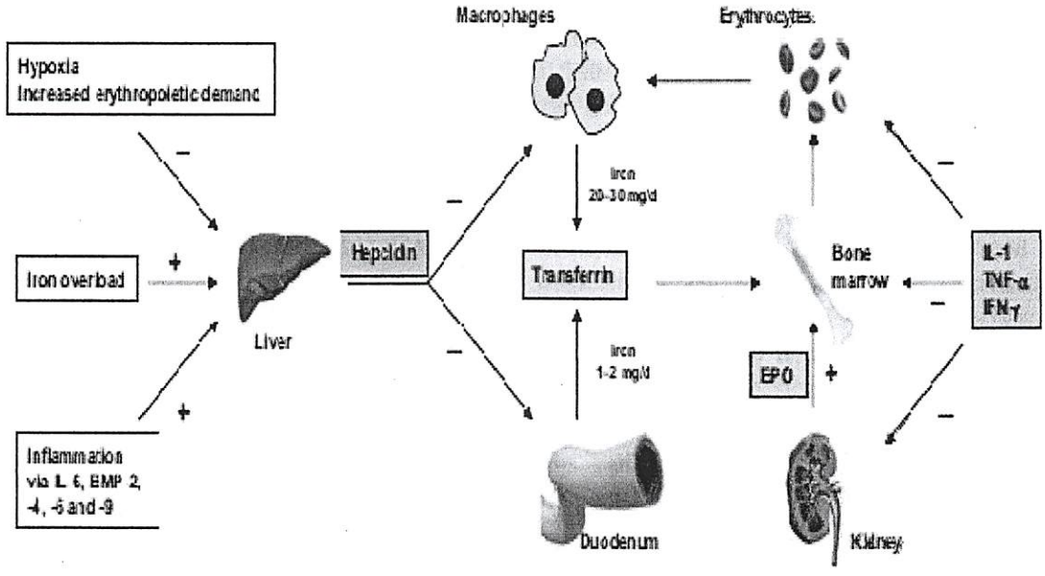


Figure 1. The effect of inflammation on iron metabolism 21)

Proinflammatory cytokines is also an antagonist of erythropoietin (EPO) by removing the inhibitory effect on erythroid progenitor cells. EPO resistance is also caused due to inflammation, with negative effects on EPO receptor. That explains why 10% of patients who received EPO hiporesponsif to treatment and require a greter dose.27)

Hepsidin, a peptide produced by the liver is the major regulator of iron balance in the body and also serves as an antimicrobial peptide that limit the **availability of iron**. Hepsidin synthesis is regulated by the body's **iron status** and triggered by IL-6 during inflammation and hepsidin-IL-6 action is responsible for the hipoferremia due to inflammation. IL-6 also triggers the production of CRP.28) Nagakawa et al study in adults in Japan, found that **low serum iron associated** with high levels of IL-6. 29)

3.C-Reactive Protein (CRP)

CRP is an acute phase protein present in normal serum, although in very small concentrations in an inflammatory reaction or tissue damage caused by non-infectious diseases and infections. CRP concentration can be increased up to 100 times. Plasma CRP is produced by hepatocytes cells mainly affected by IL-6.30) CRP is a marker of inflammation that is produced and released by the liver under .αthe stimulation of cytokines such as IL-6, IL-1 and TNF-α. CRP synthesis in the liver took place very quickly after there is little stimulation, increased serum concentrations above 5 mg / L for 6-8 hours and in peaks around 24-48 hours. The half-life in plasma is 19 hours and settled on all health and ill.31) Reference value CRP levels in healthy adult humans as measured by the method of hsCRP is <5 mg / L (0.5 mg / dL) .30) The Center for Disease Control defines CRP is a reference value 1-3 mg / L (0.1-0.3 mg / dl) .30) Systemic inflammation that occur in hemodialysis patients can be assessed by measuring the levels of pro-inflammatory biomarkers such as CRP and inflammatory cytokines such as IL6 and IL8. CRP can be increased significantly (up to 2000-fold) 4-6 hours after inflammation. During or IL-1 stimulates the *expression* of IL6αthe acute phase response, TNF thus increasing CRP gene expression in the liver resulting in an increase in the hepatic synthesis and release of CRP from the endoplasmic reticulum. 31,32,33) Xu Y et almengatakan that hepsidin levels, IL6, and hsCRP was higher in patients pre- and post-hemodialysis compared with a control group of healthy people ($p < 0.01$, $p < 0.05$ and $p < 0.01$) .34) Research conducted by Ali S, told that there is a significant relationship between CRP with indicators of renal function (creatinine clearance and urea) in patients with diabetes mellitus type 2.35) Evidens shows that inflammation is an important factor that relate **to the variability in age of erythrocytes** in patients with CKD retracts into 60-90 days, Bone marrow in non CKD patients, **will significantly improve the production of erythrocytes**, but in patients with CKD is a blunt response because of the relative EPO deficiency (Figure 2) 36) High CRP levels are **poor predictors of Hb stability control** in patients with CKD. Study suggested that **CRP is a cause of Hb variability** that can be observed. Inflammation has indirect influence on Hb variability through its **effect on iron metabolism**. 37)

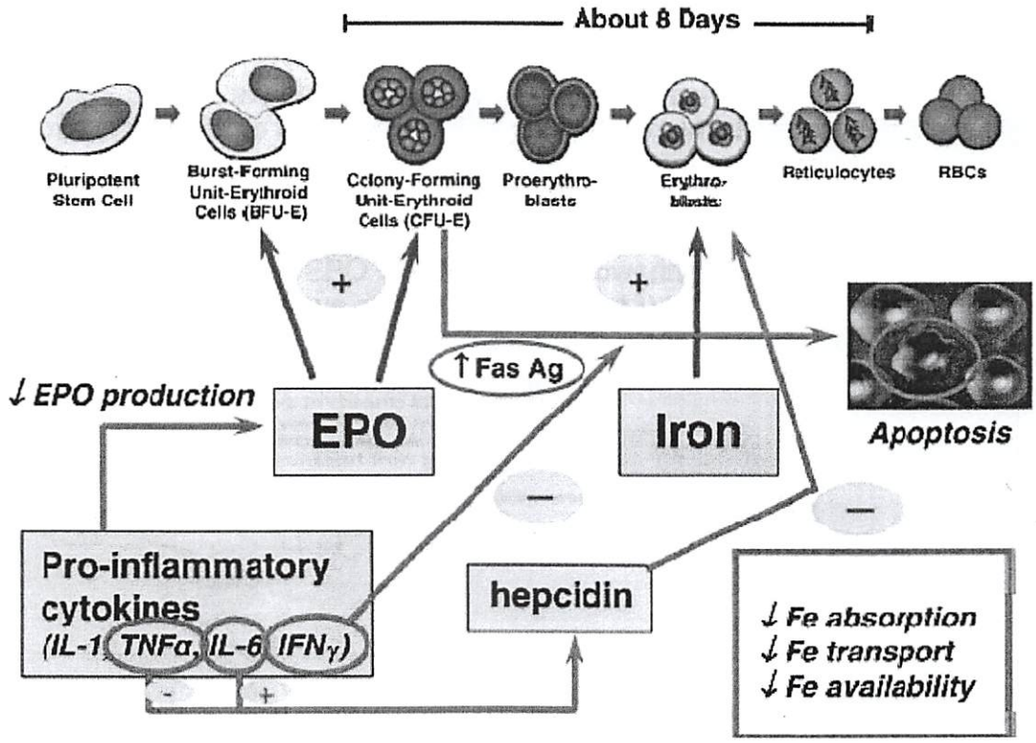


Figure 2. Eriythropoiesis in CKD. [36)]

4.Metabolisme iron in CKD patients

Hemodialysis patients experiencing iron dificiency, first because of blood loss caused by the retention of the dialysis filter, repeated *blood sampling* for laboratory examinations, and blood loss from other sources. Patients with blood loss would compensate with increased iron absorption in the intestine, but in hemodialysis patients normal iron absorption capacity or even increased.38) Loss of red blood cells in the membrane dialiser totaling 0.5 to 11 ml in a single hemodialysis (0.5 to 11 mg iron), an average of 5 ml of red blood cells (5 mg of iron), so that for one year , a patient will lose iron more than 1200 mg, more than all the reserves of iron body.39)

The second, change is due to the therapy of recombinan Human EPO (rHuEPO). RHuEPO therapy with high doses would trigger erythropoiesis, which is probably faster than the speed of erythrocyte production. Pool iron circulation (3mg) will be tired and pool iron stores are not able to release iron, offset the speed of erythropoiesis resulting **in functional iron deficiency** . Iron index showed normal serum ferritin with decreased circulating levels of iron (transferrin saturation) .40)

Third, the inflammation that occurs in hemodialysis patients take a significant effect on iron. During inflammation, hepsidin will be produced by the liver. In addition to inflammation, there is also an infection that causes increased iron requirements for

growth kuman.41) A study showed that the inflammation would disrupt the availability of iron for erythropoiesis, its shown which hemodialysis patients with CRP> 8 mg / L , iron absorption was lower than in patients with CRP < 8 mg / L.42) This above is referred to as functional iron deficiency, which is a state where the iron available is not sufficient for erythropoiesis. This is due to the blockade on RES is caused by an infection or inflammation. Infection and *inflammation* will induce the release of cytokines such as IL-1, TNF- α and IL-6. These cytokines cause a reduction in endogenous EPO production or lowering the sensitivity of erythroid precursor cells to endogenous and eksogen EPO.43)

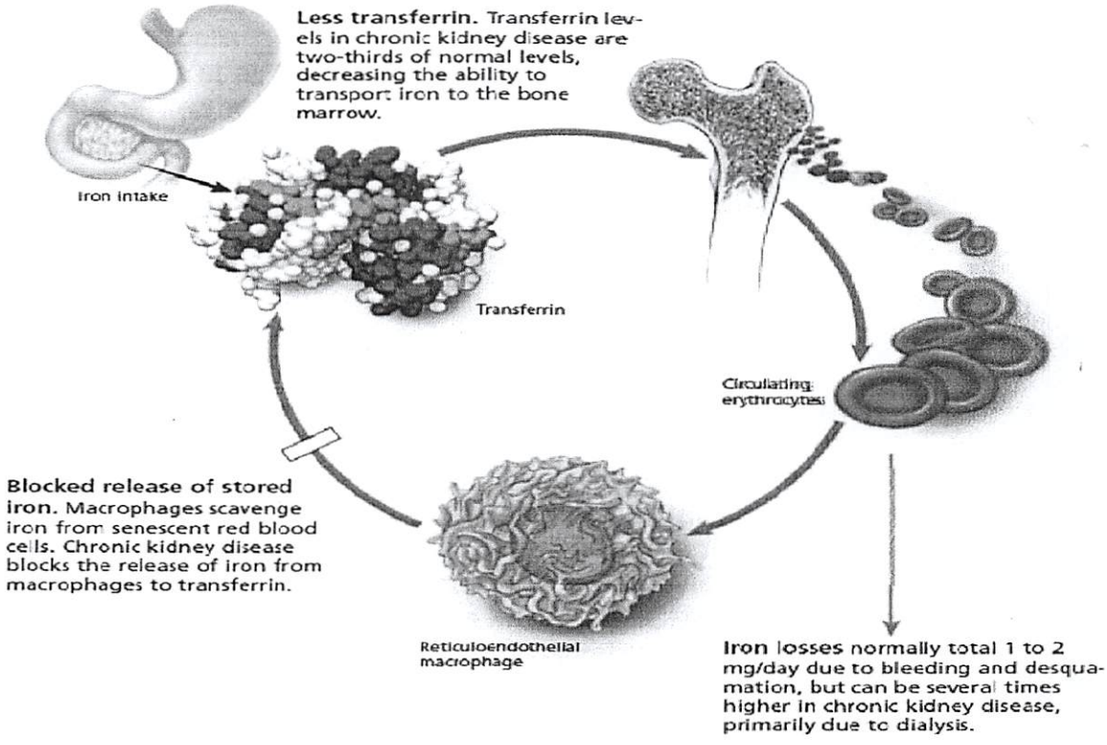


Figure 3. Metabolisme of iron in CKD patient. [44)]

Functional iron deficiency anemia is more common and strongly associated with the upregulation of inflammatory cytokines and decreases the response to erythropoietin, which would impede the transport of iron from tissues to eritroblas. Elevated levels of inflammatory cytokines such as IL-6 will trigger the production of hepsidin that would inhibit intestinal iron absorption, and lower the iron transport from the reticuloendothelial system to the bone marrow .45)

Markers of iron status in patients with CKD.

Parameters which often used to detect iron deficiency in CKD is ferritin and STfr. Serum ferritin indicates iron stores, while STfr showed iron delivery. In patients with CKD, serum ferritin levels <100μg / L indicates iron deficiency , and if serum ferritin is between 100-200 with <20% it showed there is a **functional iron deficiency**.46) Low of trasferin will inhibit iron transport to the location of the hematopoietic , in which are the causes of low **hemoglobin synthesis** and hiporesponsif in hemodialisis patients.47) Serum Ferritin is an examination which is quite expensive and is influenced by several condition such as infection, inflammation and malignancy. Other tests to diagnose iron status indirectly in the red blood cells is the percentage of hypochromic red blood cells and hemoglobin content in retikulosit.48) According to Rafi A et al, transferrin saturation and ferritin has a low sensitivity for the diagnosis of the iron status in patients with CKD undergoing HD and not good for monitoring iron therapy, necessitating new index as a Content Hemoglobin reticulocyte (CHr) and the percentage of red cells hypocromic (Hypo%) which were more sensitive, and the diagnosis of the iron status would be more accurate resulting in lower use of iron and rHuEPO. CHr parameters with hypochromic erythrocytes will add spesifisitasnya.49)

5.Content Reticulocyte Hemoglobin (CHr)

Reticulocyte parameters such as CHr is a good parameter to know status iron deficiency in situations where classical parameter fails or is too expensive, for example the presence of inflammation or malignancy, in the patient hemodialisis.50) CHr is a parameter that measures the amount of hemoglobin in reticulocytes. CHr reflect on how the amount of iron available in the bone marrow for the formation of new erythrocytes few days earlier, which is more relevant than erythrocyte hemoglobin age 60-120.51) CHr obtained from the calculation (CHr = MCVr x CHCMr), where the average MCVr is reticulocyte cell volume and average , CHCMr is reticulocyte hemoglobin concentrations , which were determined using the method optical cell-by-cell hemoglobin measurement. Reticulocytes are immature erythrocytes by age 1 to 2 days. When removed from the bone marrow, hemoglobin measurements indicate the amount of iron available for ertropoiesis. Lower Reticulocyte hemoglobin content than normal, indicate inadequate iron supply relative to the needs. The content of hemoglobin in the hemoglobin content of reticulocytes also affect the cells mature erythrocytes. CHr has been widely studied as an examination for absolute iron deficiency and functional iron deficiency, and has a high sensitivity and specificity. Limit values for iron deficiency still vary (28-30 pg) depending on the laboratory and tool digunakan.52)

CHr measurement is sensitive diagnostic tool for the early detection of iron deficiency anemia and can also be used for monitoring patients with acute infection or disease with functional iron deficiency. 65,66) Research carried Brugnara et al, said that with the cut-off CHr 27.2 pg, iron deficiency can be diagnosed with a sensitivity of 93.3% and specificity of 83.2% , and CHr is a reliable marker for cellular hemoglobin content and can be used to identify deficiencies status besi.53) Karagulle et al, suggested that CHr

is a parameter that can be used to diagnose iron deficiency with cut off 29 pg.54) CHr can be used **to identify functional iron deficiency** in patients receiving EPO therapy, but has limitations. CHr usually low in iron repleted patients with thalassemia and hemoglobinopathies which causes anemia micrositic. CHr also be increased in patients with iron deficiency, which anemia megaloblastic confounding due to the high mean corpuscular volume, due to megaloblastosis. Interpretation CHr levels should pay attention to the overall erythrocyte physiology, including the blood transfusion, iron therapy, B12 or folate deficiency, and hemoglobin analysis.55) The CHr value did not differ between the two sexes, but age affects the levels CHr especially for aged <18 years. Samples for examination CHr stable within 6 hours after pengambilan.56)

Some researchers claim that CHr is appropriate indicators to detect changes in iron status and to evaluate the efficacy of iron supplementation in the period of short time.57) CHr also have clinical benefits to determine the need for iron supplementation in patients with functional iron deficiency, eg in hemodialysis patients who receive therapy EPO. Previous research suggests that CHr <28 pg more accurate for predicting functional iron deficiency than the combination STfr and ferritin. According Vidyanshankar et al, iron screening is very useful, but in the presence of inflammation, the combination of parameters CHr with Hypo% will add more their spesificity.58) Same case according Hakick et al and Miwa et al, that the determination of reticulocyte hemoglobin level is an ideal examination of iron status in hemodialysis patients especially for the diagnosis of iron deficiency.13,59) Lin Chuang et al also stated that the change in the value CHr in 2 or 4 weeks is a better marker than conventional iron index and is a reliable parameter for detecting iron deficient erytropoiesis in hemodialysis patients during administration of eritropoietin.60) The Eropean best practice guidelines and KDOQI has set some parameters to identify and manage the deficiency in patients undergoing hemodialysis. (Table 2) 60)

Table 2. Guideline anemia, for patient undergo hemodialysis. [61)]

	European Best Practise Guideline	**KDOQI**
Target hemoglobin (g/L)	>11.0 (110)	11.0-12.0 (110-120)
Ferritin, ng/mL (pmol/L)	>100 (225)	>200 (449)
STfr (%)	>20	≥20
Hypo%	<10	-
CHr (pg)	>29	>29

The prevalence of inflammation in hemodialysis patients around 40-60%. CHr has been used as an indicator of iron deficiency and management in hemodialysis patients. Research by El Khatib et al (2006) in hemodialysis patients, showed that low CHr associated with inflammatory processes and EPO resistance and high CRP were associated with CHr rendah.61)

6. RESEARCH METODE

The study design was observational analytic with cross sectional approach (cross-sectional) ,where researchers conducted observations or measurements of variables at a given time. Each subject only observed one time and measurement variables made at the time the subject of the investigation. This research was conducted in the hemodialysis unit Dr.Kariadi Semarang and Clinical Laboratory department Dr.Kariadi Hospital Semarang for the assessment of CRP and CHr. the study time is the month of April 2015.

Samples were end-stage CKD patients undergoing hemodialysis who meet the inclusion and exclusion criteria that have been defined. Large sample studies using larger sample formula correlative analytic research, where results of previous studies linking between CRP and CHr obtained the value r=0,56.16) Value of the error type 1 (α) = 5%, so one direction based on the Z value based on table calculated as 1,64. Value of the error type 2 (β) calculated by the formula = 10%, , sample size is calculated by the formula Zβ, then the value is 1,28.73)
Sample size was calculated based on the formula:

$$n = \left\{\frac{Z\alpha+Z\beta}{0{,}5\ \ln[(1+r)/(1-r)]}\right\}^2 + 3$$

$$n = \left\{\frac{1{,}64+1{,}28}{0{,}5\ \ln[(1+0{,}5)/(1-0{,}5)]}\right\}^2 + 3$$

$$n = 31$$

Data in the form of primary data obtained from the examination of the venous blood sample of respondents. Selection of study subjects performed consecutive sampling to meet the inclusion and exclusion criteria of the study. Samples are patients with CKD who undergo hemodialysis and willing to participate in the study by signing the informed consent. CKD diagnosis is made by a specialist in internal medicine. Inclusion criteria: 1) Men and women, aged 18-55 years 2) Undergoing HD routine at least 3 months. 3) Hemodialysis conducted 2x / week, each 4 hours. Exclusion criteria: 1) Pregnancy 2) Menstruation 3) Liver Diseases (HBsAg positive, SGPT increased > 1x reference value), 4) Fever (temperature> C°37,2), 5) Serum lipemik; 6) Serum hemolysis, 7) Serum jaundice.

7.Prosedur CRP examination by the method of hsCRP

Venous blood samples were inserted into the Vacutainer without anticoagulant and sent to a laboratory without any special treatment. Samples were centrifuged to obtain serum, then examined the levels of CRP. Treatment specimen: 1) Venous blood was placed in a Vacutainer tubes without anticoagulant with the blood flow through the tube wall vacutainer.2) centrifugation immediately for 10 minutes with a speed of 3000 rpm and separate the serum. 3) Stability of specimens: 15-20months° C, 4 hours (fresh); ≤-20°C, 3 month. The level of serum CRP used Pureauto S CRP latex reagent (S-type) with Hitachi models 917 (7170) automated analyzer. The method used is latex agglutination immunoassay with *monoclonal* antibodies.

8. Examination procedure CHr:

Methods, flowcytometry, by measuring the level of fluorescence of a special coloring that bind RNA with fluorochrom. Principle of work, CHr reflect on how the amount of iron available in the bone marrow , for the formation of new erythrocytes few days earlier, which is more relevant than erythrocyte hemoglobin with age 60-120 hari.46,65) CHr obtained from the calculation (CHr = MCVr x CHCMr), where the average MCVr is reticulocyte cell volume, and average CHCMr is reticulocyte hemoglobin concentration, is determined using a method optical cell-by-cell hemoglobin measurement. Reticulocytes measured using FL1RNA and 7° light scatter.

9.Management and data analysis

Data collected includes interviews, laboratory tests. The data collected is done editing, coding, and analyzed by a computer program. Data analysis included descriptive analysis and hypothesis testing. Univariate analyzes performed on each variable to determine the characteristics of the sample. Bivariate analysis conducted to find the relationship between the relationship between CRP levels CHr in patients with end-stage CKD who undergo hemodialysis.

10.Research ethics.

Ethical clearance was obtained from the Health Research Ethics Committee of the Faculty of Medicine, University of Diponegoro / Hospital dr. Kariadi. Ethical clearance No 137 / EC / FK-RSDK / 2015. All recipients are required consent by signing a written informed consent prior to the study. Identity of research subjects kept confidential and all costs associated with the research is the responsibility of the researcher. All prospective research subjects given a full explanation of the purpose, benefits, and research procedures. Subjects have the right to refuse to participate in the study.

11.Research Results

This study was conducted in Februari-April 2015 in the Hospital hemodialysis unit Kariadi Semarang and examinations were conducted at the Laboratory of Clinical

Pathology department , Hospital dr. Kariadi. Sampling was done by consecutive sampling. Specimens taken shortly before hemodialysis during the installation of an intravenous line. Retrieved 35 regular HD patients (2 times in 1 week) which is available as a research subject. There are 4 subjects of the study were excluded because they have high levels of serum glutamic piruvic transaminase **(SGPT)** (> 1x reference value).

Characteristics of study subjects

There are 31 study subjects consisted of 19 men (61.2%) and 12 women (38.8%). The youngest age of the study subjects 26 years old and the oldest 54 years old. Since the month of February 2015 Dr Kariadi has implemented the use of single use dialysate membrane. EPO therapy given to 22 research subjects (70.9%). No subjects who received intravenous iron and blood transfusions. Data on age, duration of hemodialysis, hemoglobin levels, the number of leukocytes, CHr can be seen in Table 3. All data distribution is not normal. Statistical test to determine the normality of the data using the Shapiro-Wilk test.

Table 3. Characteristics of the study subjects

Characteristics	**n=31 (%)**	**mean±SD**	**Median (min-max)**
sex			
• men	19 (61,2%)		
• women	12 (38,8%)		
Age(years)		48,8 ± 9,01	48 (26-54)
Long HD (month)		14,3 ± 10,06	12 (3-60)
Use of EPO			
• Yes	22 (70,9%)		
• No	9 (29,1%)		
Hb (g/dl)		9,2 ± 1,74	9,2 (4,33-12,1)
The number of lekosit (thousand /mm^3)		8,3 ± 2,29	8,96(4,06-11,8)
SGPT (U/L)		43,1 ± 21,7	35 (18-97)
CHr (pg)		28,5 ± 2,4	29,5 (22,5-31,9)

CRP levels in all study subjects in this study on the value of referral Center for Disease Control (0.1-0.3 mg / dL) and 30 (96.7%) above the limit indicator of inflammation in patients receiving hemodialysis determined by NKF -KDOQI (<5-10 mg / dL). The

mean levels of CRP in this study was 3.6± 6.7 mg / dL. Most of the study subjects (13 persons, 41.9%) had CHr levels below the reference value, for hemodialysis patients (<29 pg). The mean levels of CHr is 2,4 pg.± 28,5pg. Data of CRP and CHr is not normal data distribution. Transformation of data is need, so that data into a normal distribution. Results of data transformation, data obtained CRP and CHr remained normal so Spearman correlation test was used to *analyze the data*. The relationship between serum CRP with CHr after a negative test was found negative correlation (p = 0.023 r = - 0.408). (Figure 5)

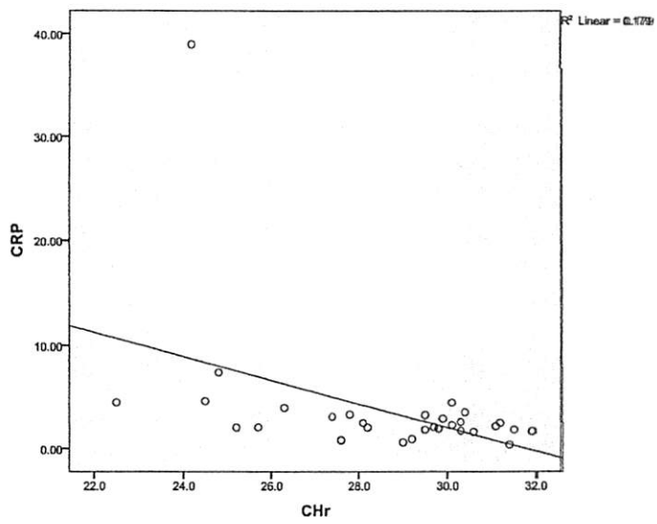


Figure 5. The graph scatter plots the relationship between CRP with CHr in CKD patients undergoing hemodialysis

12.Discussion

Comparison of the distribution of the sexes in this study more gender males than females, respectively of 19 (61.2%) male subjects and 12 (38.8%). This is similar to the results of the survey Riskesdas 2013, the prevalence of CKD in males (0.3%) higher than in women (0.2%). 62)

Age of study subjects in this study is limited between the ages of 18-55 years. Lowest subjects 26 years of age and the oldest 54 years old with a mean of 43.8± 9 years. The results are consistent with data from the 4th Report of Indonesian Renal Registry 2011, the majority of patients in the age group 45-54 years as many as 27% .63) Result of this study differs from Mitsopoulos et al with age restricted subjects > 18 years obtained a mean age of 65,1± 12,8 year.27) Similarly, research by El Khatib et al, the mean study subjects gained 54 ±12 year.64)

The mean Hb levels in this study 9,2 ±1,7 G/DL. The prevalence of anemia increases with a decrease in kidney function and often occurs when the GFR ≤ 60 MKL/MIN/1,73/m2. It has been proved by Astor B et al, in the adult population in the

US, the GFR <60 ml / min / 1.73 / m2, strongly associated with the prevalence of anemia.65) The causes of decrease in Hb levels in CKD patients is multifactorial including a deficiency of EPO , inflammation, shortening the age of erythrocytes, and iron deficiency. 65) The evaluation recommended by KDOQ, for deficiency anemia , began in Hb <12 g / dl or hematocrit <37% in men , and Hb <11 g / dl or hematocrit <33% of postmenopausal women and prepubertal patients, while consensus management of anemia in patients with chronic kidney disease, PERNEFRI in 2001 recommends an evaluation of anemia initiated Hb 10 g / dL or hematocrit of 30%. 67) The etiology of anemia in patients with CKD is a deficiency of iron, and without adequate iron stores, EPO therapy would not adekuat.68) NHANES claimed 38.3% of the 3453 anemic subjects with GFR between 20-60 ml / min / 1.73 m2 has transferrin saturation <20% .68)

Two forms of circumstances that may occur with administration of EPO therapy, namely (1) True Iron deficiency occurs during long-term administration of EPO due to the progressive transfer of iron from the body's reserves of iron deposits towards eritron. (2) Functional or relative iron deficiency occurs in the reserve state body iron stores are normal (or even increased) but the supply of iron into eritron inadequate to meet the needs of erythroid progenitor cells.(3) An imbalance of supply of iron for erythropoiesis with STfr low (<20%).
According **Testitore et al cited by Ombuh C,** therapy EPO stimulates the bone marrow to increase erythropoiesis aim of increasing the amount of hemoglobin, while the iron is one of the Hb-forming material, so when iron is available is not sufficient then EPO therapy is not beneficial and the patient will remain in status anemia.3) K / DOQI work group menyakan that absolute iron deficiency, a lack of iron in the bone marrow associated with STFR <20% and obtained the risk of iron overload if STFR> 50% .69) Penelitian **by Malyszko** et al that functional iron deficiency was found in 23% hemodialysis patients and is associated with serum iron (r = -0.59 p <0.001), STfr (r = -0.64, p <±0.001), with higher levels of serum fe 43.43 STfr±16.85 and 15.94 3,19; hsCRP 0.7 (0.02 to 1.5).70)

The mean levels of CHr which is a marker of bone marrow iron stores for28.5 ± 2.4 pg with a range of at least ± 22,5 pg, and maximum 31, 9 pg, in this study. Research subjects who have CHr <29 pg many as 13 people (41.9%). The result is lower than the results of research by **Hackeng** et al with CHr mean 31,5± 2.2 pg r (25-9 - 37,5pg), with a research subject 5 HD patients who received intravenous iron therapy. The mean levels of serum CRP was 3.6±6,7 mg/dl, with the range 0,37 mg/dl and maximum 39 mg/dl. Research by **Hackeng** et al is similar to serum CRP levels of 2.9±5,71 mg/dl (0,2-31,5 mg/dl). 25) Most of the subjects of the study had serum CRP levels> 0.5 mg / dl (30 people, 96.77%). The inflammation levels largely above the limit indicator of inflammation by NKF-KDOQI (<0,5-1mg / dl) .71)

Observations during the first trimester, patients with persistent increase in CRP has a high mortality risk. **72) Helal et al** stated CRP was an independent predictor of mortality of patients on hemodialysis (RR 1,062 p = 0:03), every increase of 0,1 / dL increases the risk of death of 6.2% .**73)** Meanwhile, according to **Racki et al**, the concentration of

CRP> 0.62 mg / dL on regular hemodialysis patients is a predictor of mortality in patients with CKD. **74)** Research in Germany, mortality increased almost 2X with CRP> 0.8 mg / dL.**75)**

One cause of inflammation in patients with HD is the use of Central Venous Catheter (CVC). CVC usage associated with high levels of CRP, *and the* data showed a decrease in CRP levels after the release of CVC. Avoiding the use of CVC is an effective strategy for reducing the inflammatory response in patients on hemodialysis kronis.76) subjects of this study partly using CVC and partly using VP shunt. The relationship between CRP with CKD CHr on the results of this study found a strong negative correlation between CRP and CHr (p = 0.023 r = - 0.408). This is consistent research **Hackeng** et al, CHr affected by inflammation, which found a negative correlation between CHr and logCRP (rho -0.5 p <0.0001). The study also correspond **El Khatib** et al, found the differences between the groups CHr CRP levels <0.5 mg / dl and CRP> 0.5 mg / dl (p, 0.005).

Limitations of the study: 1) not examined parameters deficiency anemia besi.2) This study does not distinguish or separate the VP shunt and CVC use for hemodialisis.3) This study did not distinguish between subjects who received EPO therapy and who did not receive EPO therapy so that the effect of the CHr not known.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions: There is a negative correlation between CRP with CHr in hemodialysis patients Suggestion: Further studies should be done with due regard to EPO therapy and intravenous iron administration, having regard to factors such as the use of inflammatory originator VP shunt or CVC in hemodialysis patients and anemia status.

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This is to certify that

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has participated as a

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in

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Held in Medan, Indonesia, 5-6 September 2015

DR. dr. Djumhana Atmakusuma, SpPD-KHOM
National President of The Indonesian .
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Prof. Shinichiro Okamoto, MD, Ph.D
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