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## Article Categories

### C-reactive Protein and Hepsidin in Non-Dialysis Chronic Kidney Disease

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#### ABSTRACT

Complications such as anemia and its clinical consequences arise as chronic kidney diseases progress. One renal anemia pathophysiology is a disruption of iron metabolism, regulated by the main iron exporter hormone, hepcidin. Chronic kidney disease patients were constantly in an inflammatory state, represented by an increased in C-reactive protein. This inflammatory state would facilitate the liver to secrete hepcidin, which would subsequently follow a decrease of iron circulation, thus resulting in functional iron deficiency. Both acute phase reactants which used thoroughly as markers in tropical and infectious diseases, had their own roles in chronic kidney disease. The correlation of c-reactive protein and hepcidin in chronic kidney disease patients was still controversial. To analyse the relationship between c-reactive protein and hepcidin in non-dialysis chronic kidney disease patients. We conducted an observational cross-sectional study with 40 non-dialysis chronic kidney disease patients who met the inclusion and exclusion criteria. Patients were enrolled with consecutive sampling and were examined for serum c-reactive protein and hepcidin levels. A total of forty subjects (67.5% male with mean age of  $50.23 \pm 1.04$  years) were eligible for enrolment in this study. The most comorbid factor was hypertension (62.5%). The common stage for chronic kidney disease was stage 3 (40%). The mean hemoglobin value was  $10.74 \pm 0.36$  g/dL, mean blood urea nitrogen was  $39.98 \pm 29.59$  mg/dL, and serum creatinine of  $4.12 \pm 3.39$  mg/dL. Mean serum c-reactive protein levels were  $3.52 \pm 5.13$  mg/l. Mean hepcidin level were  $94,03 \pm 95,39$  ng/ml. Serum C-reactive protein levels correlated positively ( $r=0.487$ ) and significantly ( $p$ -value=0.001) with serum hepcidin value. C-reactive protein and hepcidin was significantly correlated in non-dialysis chronic kidney disease patients.

**Keywords:** CRP; Hepsidin; CKD; non-dialysis; iron; liver

#### ABSTRAK

Progresivitas penyakit ginjal kronis akan membawa komplikasi anemia dengan berbagai konsekuensi klinis. Salah satu patofisiologi anemia pada penyakit ginjal kronis dapat diakibatkan oleh gangguan metabolisme besi yang diatur oleh hormon eksporter utama besi yaitu hepsidin. Pasien penyakit ginjal kronis berada dalam kondisi inflamasi, yang diwakili dengan peningkatan c-reactive protein. Adanya inflamasi akan menyebabkan liver mensekresi hepsidin yang kemudian berdampak pada menurunnya kadar besi dalam sirkulasi yang dapat berdampak pada anemia defisiensi besi fungsional. Kedua reaktan fase akut yang biasa digunakan dalam penyakit tropik dan infeksi, ternyata juga memiliki peran dalam penyakit ginjal kronis. Hubungan antara c-reactive protein dengan hepsidin pada penderita penyakit ginjal kronis non-dialisis masih menjadi kontroversi. Menganalisis hubungan antara kadar c-reactive protein dengan hepsidin pada pasien penyakit ginjal kronis yang belum menjalani hemodialisis. Studi ini adalah studi analisis observasional cross sectional, diikuti 40 pasien penyakit ginjal kronis yang belum menjalani dialisis yang sesuai dengan kriteria inklusi dan eksklusi. Subjek penelitian di ambil secara konsekutif dan diperiksa kadar c-reactive protein serum dan hepsidin serum. Empat puluh subjek penelitian ini, terdiri dari 27 subjek laki-laki dan 13 subjek perempuan dengan rerata usia 50,23 tahun. Penyakit komorbid terbanyak adalah hipertensi (62,5%). Stadium terbanyak adalah stadium 3. Rerata kadar hemoglobin pada penelitian ini sebesar  $10,74 \pm 0,36$  g/dL, rerata blood urea nitrogen  $39,98 \pm 29,59$  mg/dL, dan rerata serum kreatinin sebesar  $4,12 \pm 3,39$  mg/dL. Rerata kadar c-reactive protein serum sebesar  $3,52 \pm 5,13$  mg/l. Rerata kadar hepsidin serum sebesar  $94,03 \pm 95,39$  ng/ml. Pada

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penelitian ini diperoleh hubungan positif ( $r=0,487$ ) yang signifikan ( $p=0,001$ ) antara c-reactive protein dan hepsidin. Didapatkan hubungan positif yang signifikan antara kadar C-reactive protein dan hepsidin pada pasien penyakit ginjal kronis non-dialisis.

**Kata kunci:** CRP; hepsidin; PGK; non-dialisis; besi; liver

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## INTRODUCTION

Hepsidin and c-reactive protein (CRP) had their roles in infectious diseases for a period of time. Hepsidin lowered mammal's blood iron levels at the time the pathogen-infected the hosts. Low blood iron level hindered pathogen's growth so that infections might be stopped. C-reactive protein had its own roles in activating platelets, leukocytes, endothelial growth factors, complements and chemokines during infections to cease infection. However, the roles of these two markers in renal anemia in chronic kidney disease (CKD) have not been elucidated yet.

C-reactive protein has been one of the sensitive inflammation markers which correlate with hepsidin in CKD. There have been substantial studies to backed up and come against it. Chronic kidney disease, stated as a chronic state of low-grade inflammation, could initiate a chain of sequences that lead to secretion of CRP and hepsidin. However, hepsidin was first recognized by Ganz, et al. as liver expressed antimicrobial peptide-1 (LEAP-1) secreted during infection or high-grade inflammation, putting hepsidin into lower place in this chain of sequences than CRP.

C-reactive protein was proven to be inversely correlated with the estimated glomerular filtration rate (eGFR) and stage in CKD. CRP also correlated with other inflammation markers such as interleukin-6 (IL-6). Interleukin-6 was directly correlated with the secretion of CRP and hepsidin in the human liver.

Hepsidin is a major iron exporter hormone in mammals. It interacts with its receptor, ferroportin in gastrointestinal tracts and reticuloendothelial systems. Degradation and internalizing process of ferroportin inhibits daily iron intake entering

circulation from duodenum and traps intracellular storage iron. These processes create a hypoferremia state which results in functional iron deficiency anemia. Anemia brings clinical consequences such as a decrease of quality of life, deterioration of eGFR, increased cardiovascular events, increased mortality rate, and even increased economical burden. High inflammation state and other confounding factors (anemia, duration of dialysis) was seen in CKD patients on dialysis which lead to sample selection of non-dialysis patients.

## MATERIALS AND METHODS

**Study design:** This was an analytic observational study with cross-sectional design in CKD patients in Nephrology Outpatient Clinic at Dr. Soetomo General Hospital, Surabaya, Indonesia. This research was ethically approved by Health Research Ethics Committee of Dr. Soetomo Hospital. Written informed consent was obtained from all subjects. Chronic kidney disease, diagnosed using KDIGO criteria, are abnormalities in kidney function or structure that have occurred for more than 3 months. The stage was determined based on the decrease in eGFR with the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula.<sup>1,2</sup> Inclusion criterias for the samples were non-dialysis stadium III-V chronic kidney disease patients. Patients with history of cancer, hepatitis B, hepatitis C, liver cirrhosis, diabetes mellitus, chronic inflammation (HIV-AIDS, obesity, rheumatic disease, geriatric patients), diagnosed with acute infection (urinary tract infection, respiratory infection, pneumonia, gastroenteritis), under oral and intravenous iron or erythropoietin

stimulating agents (ESA) therapy, hormonal therapy, history of blood transfusion, alcoholism, absolute iron deficiency, gastrointestinal bleeding were excluded.<sup>1</sup>

**Data collection:** Consecutive sampling was done to complete an amount of 40 samples. Direct interview was done by the author and blood samples were taken by professional healthcare and sent to Dr. Soetomo General Hospital laboratory to be examined. Inflammations in this research were represented by serum CRP. Serum level of CRP was measured using *extended range C-reactive Protein* method with reagent *Siemens Flex® Reagent Cartridge C-reactive Protein Extended Range CAT No. RCRP-3749*, an in vitro diagnostic test with a particle enhanced turbidimetric immunoassay (PETIA) technique, meant to quantitatively measure CRP level in human serum. Serum level of hepcidin was circulating level of hepcidin-25 in blood. Serum hepcidin level was measured using the Enzyme Linked Immunosorbent Assay (ELISA) method. Serum were stored in a deep freezer at a temperature of  $-80^{\circ}\text{C}$  until the hepcidin measurement was performed. The reagent used was DRG Hepcidin-25 (bioactive) ELISA from *CAT No EIA-5782*, an enzyme immunoassay for in-vitro quantitative examination for hepcidin-25 peptide in serum and plasma.<sup>3,4</sup>

**Statistical analysis:** All data was analysed by Statistical Package for the Social Sciences (SPSS ver 23). Data was delivered in the form of analytic statistics. Data analysis was provided in mean  $\pm$  standard error of mean (SE). Correlation of serum hepcidin with CKD stage was calculated by Pearson parametric test if it had normal distribution or Spearman parametric test if the data distribution was not normal. It was said to be significant if the p-value is  $<0.05$ .

## RESULTS AND DISCUSSION

### Patient Characteristics

A total of forty subjects (67.5% male with mean age of  $50.23 \pm 1.04$  years) were eligible for enrollment in this study. This study was done in Nephrology Outpatient Clinic, Dr Soetomo

**Table 1.** Demographic Characteristics

| Category                      | Result                             | Frequency  |
|-------------------------------|------------------------------------|------------|
| <b>Sex</b>                    |                                    |            |
| Male                          |                                    | 27 (67.5%) |
| Female                        |                                    | 13 (32.5%) |
| <b>Age (years)</b>            |                                    |            |
| Mean $\pm$ SE                 | <b>50.23 <math>\pm</math> 1.04</b> |            |
| Range (Min - Max)             | <b>27–58</b>                       |            |
| <b>BMI (kg/m<sup>2</sup>)</b> |                                    |            |
| Mean $\pm$ SE                 | <b>20.54 <math>\pm</math> 0.58</b> |            |
| Range (Min - Max)             | <b>14.57 – 24.38</b>               |            |
| <b>CKD Stage</b>              |                                    |            |
| Stage 3                       |                                    | 16 (40%)   |
| Stage 4                       |                                    | 9 (22.5%)  |
| Stage 5                       |                                    | 15 (37.5%) |

General Hospital, Surabaya, Indonesia within the period of 1 June 2018 - 31 August 2018. The results of demographic and clinical characteristics of this study subjects were described in Table 1 and Table 2.

Twenty seven of 40 subjects were male (67.5%), the youngest is 27 years old and the oldest is 58 years old with mean age of 50.23 years old (SE 1.04), mean of body mass index (BMI) was 22.54 (SE 0.57). Based on CKD stage, 16 patients (40%) of the total sample had stage 3 CKD. (Table 1)

**Table 2.** Clinical characteristics

| Clinical Data                   | Level                              | Frequency        |
|---------------------------------|------------------------------------|------------------|
| <b>Hemoglobin (g/dL)</b>        |                                    |                  |
| Mean $\pm$ SE                   | <b>10.74 <math>\pm</math> 0.36</b> |                  |
| Range (Min - Max)               | <b>7.30 – 15.70</b>                |                  |
| <b>BUN (mg/dL)</b>              |                                    |                  |
| Mean $\pm$ SE                   | <b>39.98 <math>\pm</math> 4.68</b> |                  |
| Range (Min - Max)               | <b>11 – 125</b>                    |                  |
| <b>Creatinine serum (mg/dL)</b> |                                    |                  |
| Mean $\pm$ SE                   | <b>4.12 <math>\pm</math> 0.54</b>  |                  |
| Range (Min - Max)               | <b>1.22 – 16.53</b>                |                  |
| <b>Comorbid disease</b>         |                                    |                  |
| Hypertension (n)                |                                    | 25 (62.5%)       |
| Urinary tract stone (n)         |                                    | <b>7 (17.5%)</b> |
| Others                          |                                    | 8 (20.0%)        |

**Table 3.** CRP level characteristics

| Stage   | CRP level (mg/L) |        |                 | p     |
|---------|------------------|--------|-----------------|-------|
|         | Mean ± SE        | Median | Range (Min-Max) |       |
| Stage 3 | 1.66 ± 0.41      | 0.25   | 0.10 – 9.40     | 0.036 |
| Stage 4 | 2.68 ± 0.49      | 1.10   | 0.10 – 8.90     |       |
| Stage 5 | 6.01 ± 1.11      | 2.50   | 0.30 – 21.10    |       |
| Total   | 3.52 ± 0.81      | 1.10   | 0.10 – 21.10    |       |

**Table 4.** Hepcidin level characteristics

| Stage   | Hepcidin level (ng/ml) |        |                   | p     |
|---------|------------------------|--------|-------------------|-------|
|         | Mean ± SE              | Median | Range (Min - Max) |       |
| Stage 3 | 27.24 ± 3.24           | 23.18  | 0.12 – 70.14      | 0.000 |
| Stage 4 | 84.69 ± 12.23          | 55.65  | 1.08 – 254.87     |       |
| Stage 5 | 170.88 ± 15.81         | 200.00 | 9.96 – 352.42     |       |
| Total   | 94.03 ± 15.08          | 53.98  | 0.12 – 352.42     |       |

### Clinical Characteristics

Mean hemoglobin level of 40 subjects was 10.74 g/dL with SE of 0.36. Mean of blood urea nitrogen (BUN) levels was 39.98 mg/dL with SE of 4.68. Mean level of serum creatinine was 4.12 with SE of 0.54. The most frequent comorbid factor was hypertension (62.5%) (Table 2).

Distribution of CRP levels by CKD stage results were mean CRP 166 mg/L (range 0.10 - 9.40), 2.68 mg/L (range 0.10 - 8.90), 6.01 mg/L (range of 0.30 - 21.10) in stage 3, 4, and 5 respectively. The overall mean of CRP levels in this study was 3.52 mg/L with a range 0.10 - 21.10. (Table 3)

Distribution of hepcidin value by CKD stage were mean hepcidin 27.24 ng/ml (range 0.12 - 70.14), 84.69 ng/ml (range 1.08 - 254.87), and 170.88 ng/ml (range 9.96 - 352.42) in stage 3, 4, and 5 respectively. Hepcidin level overall mean was 94.03 ng/ml (range 0.12 - 352.42). (Table 4)

### Significance and Strength of CRP and Hepcidin Value Correlation in Non-Dialysis CKD Patients.

Distribution of CRP and hepcidin level data were used to analyze the correlation between CRP and hepcidin in CKD patients. *Kolmogorov-Smirnov* normality test showed that the distribution

**Table 5.** Result of Spearman correlation test

| Variable 1 | Variable 2 | $r_s$ | p     |
|------------|------------|-------|-------|
| CRP        | Hepcidin   | 0.487 | 0.001 |

of CRP and hepcidin level data is abnormal (both p-value < 0.05). Spearman correlation test was used to further analyze the correlation between CRP and hepcidin levels. (Table 5)

Analysis of CRP and hepcidin value showed an association with a positive correlation coefficient of 0.487. The correlation of CRP and hepcidin value in this study was significant, indicated by the p-value = 0.001. The meaning of this positive correlation coefficient showed a unidirectional relation, if the CRP level increase, the hepcidin level would be increased consequently.

### DISCUSSION

Chronic kidney diseases progressed alongside complications such as anemia and its clinical consequences. One of the renal anemia pathophysiology was disruption of iron metabolism, regulated by main iron exporter hormone, hepcidin. Chronic kidney disease patients were constantly in an inflammatory state, represented by increased of CRP. This inflammatory state results in the liver secreting hepcidin, which subsequently followed a decrease in iron circulation, thus resulting in functional iron deficiency. Inclusion of stage 3 to 5 CKD patients was based on earlier studies that stated complications of CKD, particularly anemia, were more commonly seen in stage 3 to 5 CKD patients. Non-dialysis CKD patients were selected to reduce confounding factor such as duration of dialysis in CKD patients.<sup>5,6</sup>

Most of the study subjects were men with a percentage of 67.5%, similar to studies by Toima, et al., Mercadel, et al, Elmenyaw, et al.<sup>5,6,7</sup> Higher male prevalence than female could be influenced by numerous factors like hypertension, hyperglycemia, lifestyle, kidney structure and hormonal differences.<sup>8</sup>

The mean age in this study was 50.23 ± 1.04 years old, similar to studies by Mercadel, et al,

Toima, et al., 2010, Elmenyawi, et al.<sup>5,6,7</sup> Aging process influenced CKD progression and lesser function was expected from older nephrons.<sup>9</sup>

Mean hemoglobin result in this study was 10.74 g/dL with SE of 0.36. The results of this study were similar to other studies by Toima, et al., Peters, et al., and Goyal, et al.<sup>5,10,11</sup> Mean results of BUN value were 39.98 mg/dL with SE of 4.8. BUN and creatinine serum levels found in this study were similar to study by Toima, et al.<sup>5</sup>

The most frequent comorbid disease in this study was hypertension, at 62.5% of the total subjects. Study by Toima, et al., Peters, et al., and Goyal, et al., also mentioned hypertension as the most frequent comorbid disease found in CKD patient.<sup>5,10,11</sup> Hypertension was the highest prevalent chronic disease in Indonesia based on 2013 RISKESDAS study.<sup>12</sup> Hypertension risk factors were age, race, family history, obesity, high sodium intake, and smoking.<sup>8</sup>

In this study, higher mean CRP levels was seen in more advanced CKD stage. The mean total CRP level in this study was 3.52 mg/L with a SE of 0.81. This was similar to previous studies by Toima et al. who found CRP levels of 6.0 mg/L with a standard deviation of 0.9, Elmenyawi et al. who found mean CRP level was 4.28 mg/L with a standard deviation of 3.7, Rasheed et al. who found CRP mean levels were 7.59 mg/L in all CKD stages, and.<sup>5,7,13</sup> Fluctuations in CRP levels may also have been due to the highest staging differences in the population study. In a study by Elmenyawi et al the most frequent CKD stage in the population was stage 3 with mean CRP level at 3.52 and 4.28 mg/L.<sup>7</sup> In the study of Toima et al. and Rasheed et al. the highest staging in the population was stage 5 and mean CRP levels were, 6.0 and 7.59 mg/L.<sup>5,13</sup> This study found that CRP level increased along with a decrease in eGFR, which were consistent with other studies.<sup>14,16</sup>

Total mean hepcidin found in this study was 94.03 ng/ml with SE of 15.08. While Toima, et al. found mean hepcidin level of 84 ng/mL with a standard deviation of 18.6, Goyal, et al. and Uehata, et al. found mean hepcidin levels of 65.0 ng/mL and 15.4 ng/mL respectively.<sup>5, 11,17</sup>

Analysis of CRP and hepcidin levels in non-dialysis CKD patients revealed a moderate to significant relationship (correlation coefficient 0.487; p-value 0.001). This result indicated that an increase in CRP levels would lead to a directly proportional increase of hepcidin value. The results of this study were in accordance with studies by Toima et al., Peters et al., and Lee et al., who inferred a positive relationship between CRP and hepcidin levels.<sup>5,10,15</sup> Toima et al. organized a study in Egypt regarding the importance of hepcidin role as a novel biomarker which reflected iron status in CKD patients and its relationship with CRP levels. Thirty CKD patients and 10 healthy subjects, used as controls, were enrolled. The result showed a correlation in CRP and hepcidin value with R of 0.68 (p = 0.001).<sup>5</sup> Patients who had iron or erythropoietin therapy for the previous 21 days were excluded. Inclusion of diabetes mellitus patients might lead to a strong correlation found in this study. This study used the same method in CRP and hepcidin level measurement as Toima, et al.<sup>5</sup> Peters, et al. conducted an observational cross-sectional study of factors affecting hepcidin in 83 non-dialysis CKD patients and 48 dialysis CKD patients in the Netherlands. There was a weak positive relationship (r=0.21, p <0.001) between CRP and hepcidin levels which was probably related to inclusion of patients who were under erythropoietin therapy. The method used to measure CRP level was the same as in our study, but a different method (light chromatography mass spectrometry / LC-MS) was used in measuring hepcidin level.<sup>10</sup> Lee, et al. in Korea analysed whether hepcidin was a novel uremic toxin using multivariate analysis of various variables affecting hepcidin in 2090 non-dialysis CKD patients. They found a positive correlation between CRP and hepcidin with r=0.23 (p <0.001). Patients with intravenous iron, oral iron, and erythropoietin therapy were not excluded. These factors might have played a role as confounding factors to the weak correlation. This study used the same method in CRP and hepcidin level measurement as Lee, et al.<sup>15</sup>

The result of the Uehata et al. study result was different compared to this study. That study

included 505 samples of non-dialysis CKD patients and found no association between CRP and hepcidin levels ( $r = 0.03$  and  $p = 0.4$ ). Patients with liver cirrhosis were not excluded, while liver cirrhosis can induce negative feedback on hepcidin and CRP. The Level of CRP was measured using immunoagglutination detection method and hepcidin level was determined using LC-MS.<sup>17</sup> Another study by Goyal, et al. in India analyzed the relation between CRP and hepcidin levels in 100 non-dialysis CKD patients. They found similar results with this study, the correlation coefficient of 0.0001 and  $p = 0.896$  between CRP and hepcidin levels. Patients having oral iron therapy were not excluded, while oral iron could induce positive feedback on hepcidin. C-reactive protein levels were measured using different EIA kits which could influence the absence of association of CRP and hepcidin.<sup>11</sup> Study by Wagner, et al., which analysed predictive factors of mortality in patients with non-dialysis CKD patients, showed contradiction to this study by stating that CRP level was not associated with hepcidin levels (correlation coefficient of 0.01 and  $p < 0.001$ ). Their case control study stated that CRP and hepcidin (measured hepcidin using RIA method) were influenced by factors that change over time. The mean hemoglobin in their study was higher than this study (13.1 g/dL) while anemia could induce negative feedback on hepcidin.<sup>18</sup> These factors might have played a role in the absence of a correlation. Another study contrasting this study results was Macdougall et al. in the Netherlands who used a random sampling system and including patients with erythropoietin therapy which caused positive feedback on hepcidin.<sup>19</sup>

There were differences in the results of this study compared to previous studies. Different methods in measuring CRP and hepcidin levels could have contributed to this result. In earlier studies, the CRP level was measured using the immunoturbidimetric assay, immunoagglutination, or EIA method.<sup>5,10,15</sup> In previous studies, hepcidin level was measured by ELISA and LC-MS methods.<sup>20</sup> Studies conducted by Mercadel, et al., Macdougall, et al. used different methods to

determine hepcidin levels.<sup>6,21</sup> Hepcidin could be measured using RIA, ELISA, and mass spectrometry-based methods.<sup>22</sup> Measurement using RIA detects hepcidin-25 greater than actual condition. Measuring hepcidin-25 using ELISA were accurate and cheap.<sup>3,23</sup> Mass spectrometry-based was indeed more accurate but not practical, requiring more instruments and too expensive. Besides differences in measurement methods, there were also differences in this study subjects' characteristics compared to previous studies.<sup>24</sup> History of erythropoietin therapy, a history of blood transfusion, iron therapy, diabetes mellitus, and other factors influencing CRP and hepcidin level were not excluded in previous studies, whereas it could have affected the results.<sup>6,11,14,25</sup>

This was a novel study of hepcidin and inflammation marker in CKD in Surabaya, although we were aware that the small number of subjects might interfere with the study results. Study with a larger, more homogenous sample, more markers of inflammation and iron might be needed in the future.

## CONCLUSION

A significant positive correlation with  $r_s = 0.487$ ,  $p = 0.001$  was found between CRP and hepcidin levels in non-dialysis CKD patients. If there was an increase in serum CRP levels in non-dialysis CKD patients there was a tendency for an increase in serum hepcidin levels.

## CONFLICT OF INTEREST

There is no conflict of interest of this paper.

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## REFERENCES

1. KDIGO, 2012. Kidney Disease: Improving Global Outcomes (KDIGO) Clinical Practice Guidelines for Anemia in Chronic Kidney Disease. *Kidney International Supplements*, 2(4), pp. 279-335.
2. KDIGO, 2013. Kidney Disease: Improving Global Outcomes (KDIGO) Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. *Kidney International Supplements*, Volume 3, pp. 1-150.
3. Kemna, E., Tjalsma, H., Podust, V. & Swinkels, D., 2007. Mass spectrometry-based hepcidin measurements in serum and urine: analytical aspects and clinical implications. *Clinical Chemistry*, 53(4), pp. 620-628.
4. Swinkels, D., Jorna, A. & Raymakers, R., 2007. Synopsis of the Dutch multidisciplinary guideline for the diagnosis and treatment of hereditary haemochromatosis. *The Netherlands Journal of Medicine*, 65(11), pp. 452-455.
5. Toima, S., Madkour, M., Saleh, A. & Hammam, O., 2010. Hepcidin and iron status in chronic kidney disease. *African Journal of Nephrology*, Volume 14, pp. 16-23.
6. Mercadel, L., Metzger, M., Haymann, J. & Thervet, E., 2014. The Relation of Hepcidin to Iron Disorders, Inflammation and Hemoglobin in Chronic Kidney Disease. *Plos One*, 9(6), pp. 1-7.
7. Elmenyawi, A., Hassan, A. & Sawar, S., 2017. Relationship Between Hepcidin, Ferritin and C-reactive Protein in Hemodialysis Patients. *The Egyptian Journal of Hospital Medicine*, 69(2), pp. 1786-1793.
8. Chang, P., Chien, L. & Chiou, H., 2016. Risk factors of gender for renal progression in patients with early chronic kidney disease. *Medicine*, 95(30), pp. 1-7.
9. Wang, X., Liu, S. & Jin, Y., 2014. Correlation of serum high-sensitivity C-reactive protein and interleukin-6 in patients with acute coronary syndrome. *Genetics and Molecular Research*, 13(2), pp. 4260-4266.
10. Peters, H., Laarakkers, C. & Swinkels, D., 2010. Serum hepcidin-25 levels in patients with chronic kidney disease are independent of glomerular filtration rate. *Nephrology Dialysis Transplantation*, Volume 25, pp. 848-853.
11. Goyal, H., Mohanty, S. & Sharma, M., 2017. Study of anemia in nondialysis dependent chronic kidney disease with special reference to serum hepcidin. *Indian Journal of Nephrology*, 27(1), pp. 44-50.
12. Trihono, 2013. *Riset Kesehatan Dasar*. Jakarta: Badan penelitian dan pengembangan kesehatan.
13. Rasheed, N., Ali, S. & Shami, A., 2013. Serum Hepcidin Levels in Anemia of Chronic Kidney Diseases Compared to Iron Deficiency Anemia and It's Correlation with Serum Levels of Hs -C Reactive Protein, Interlukin-6 and Ferritin. *Global Journal of Bio-Science and Technology*, 2(1), pp. 43-50.
14. Nand, N., Aggarwal, H., Yadav, R. & Gupta, A., 2009. Role of High-sensitivity C - reactive Protein as a Marker of Inflammation in Pre-dialysis Patients of Chronic Renal Failure. *The Journal, Indian Academy of Clinical Medicine*, 10(1), pp. 18-22.
15. Lee, S., Kim, J. & Sung, S., 2017. Serum hepcidin may be a novel uremic toxin, which might be related to erythropoietin resistance. *Nature Scientific Reports*, Volume 7, pp. 1-7.
16. Kumar, S., 2015. Comparative Study of HS-CRP in Chronic Kidney Disease. *International Organization of Scientific Research Journal Of Pharmacy*, 5(7), pp. 8-12.
17. Uehata, T., Tomosugi, N. & Tsubakihara, Y., 2012. Serum hepcidin-25 levels and anemia in non-dialysis chronic kidney disease patients: a cross-sectional study. *Nephrology Dialysis Transplantation*, Volume 27, pp. 1076-1083.
18. Wagner, M., Ashby, D. & Schramm, L., 2015. Hepcidin-25 in Diabetic Chronic Kidney Disease Is Predictive for Mortality and Progression to End Stage Renal Disease. *Plos One*, 10(4), pp. 1-14.
19. Putten, K., Jie, K. & Gaillard, C., 2010. Hepcidin-25 is a marker of the response rather than resistance to exogenous erythropoietin in chronic kidney disease/ chronic heart failure patients. *European Journal of Heart Failure*, Volume 12, pp. 943-950.
20. Babitt, J. & Lin, H., 2010. Molecular Mechanisms of Hepcidin Regulation: Implications for the Anemia of CKD. *American Journal of Kidney Diseases*, 55(4), pp.726-41.
21. Macdougall, I., Malyszko, J. & Hider, R., 2010. Current Status of the Measurement of Blood Hepcidin Levels in Chronic Kidney Disease. *Clinical Journal of American Society of Nephrology*, 5(9), pp. 1681-1689.
22. Arezes, J. & Nemeth, E., 2015. Hepcidin and Iron Disorders: New Biology and Clinical Approaches. *Int. Jnl. Lab. Hem.*, 37(1), pp.92-98.
23. Jairam, A. *et al.*, 2010. Iron Status, inflammation, and hepcidin in ESRD patients: The Confounding Role of intravenous iron therapy. *Indian Journal of Nephrology*, 20(3), pp.125-31.
24. Kato, A., 2010. Increased hepcidin-25 and erythropoietin responsiveness in patients with cardio-renal anemia syndrome. *Future Cardiology*, 6(6), pp.769-71.
25. Miura, K. *et al.*, 2008. Hepatitis virus induced oxidative stress suppresses hepcidin expression through increased histone deacetylase activity. *Hepatology*, 48, pp.1420-29.