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RESEARCH ARTICLE

Comparative Analysis of Hepcidin-25 and Inflammatory Markers in Patients with Chronic Kidney Disease with and without Anemia

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Introduction: Hepcidin is a regulatory protein in iron metabolism; we do not know the role in chronic kidney disease anemia. **Methods**: 22 patients with CKD anemia and 15 patients with CKD without anemia were investigated. CKD anemia-inclusion criteria: over 18 years, hemoglobin ≤12 g/dl for women and ≤13 g/dl for men, no treatment for anemia 6 months before enrollment, glomerular filtration rate (eGFR) <60 ml/min/1.73m^2 and stable creatinine three months before enrollment. Exclusion criteria: infection, bleeding, malignancy, systemic or liver disease, immunosuppression, renal replacement therapy. CKD without anemia-inclusion criteria: over 18 years, no anemia or treatment for anemia, CKD with stable creatinine values three months before enrollment. Exclusion criteria: medical conditions known to have a role in the development of polycythemia. Hepcidin-25 and ferritin were measured by ELISA method. Erythropoietin (EPO), tumor necrosis factor (TNF)-α, interleukin (IL)-6 were evaluated using chemiluminescent enzyme immunometric assays. Unpaired T test, Pearson correlation and multiple regression were used for statistical analysis. **Results**: Hemoglobin values were significantly lower in anemia group. There were no differences in terms of eGFR, age, body mass index, serum hepcidin, erythropoietin, fibrinogen, IL-6, and TNF-α between CKD patients with and without anemia. Serum hepcidin correlated positively with ferritin (r=0.45 p<0.05), TNF-α (r=0.54, p<0.05) and negatively with erythropoietin (r=-0.51, p<0.05). Multiple linear regression analysis demonstrated that TNF-α is an independent predictor of serum hepcidin in our patients (p=0.003, R=0.71). **Conclusion**: We found no differences in serum hepcidin, erythropoietin and inflammatory markers in non-dialysis CKD patients with and without anemia.

Keywords: hepcidin-25, TNF-α, IL-6, erythropoietin, anemia, non-dialysis chronic kidney disease

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Introduction

Anemia is a common problem in chronic kidney disease (CKD) patient. Previous studies demonstrated prevalence rates of anemia in CKD up to 47.7% and that glomerular filtration rate, age, gender, race, comorbidities are predictors of CKD anemia. [1,2,3]

Most authors postulated that the prevalence of anemia increases with decreasing glomerular filtration rate in both genders. [4,5]

CKD anemia is a multifactorial process. Erythropoietin deficiency, iron metabolism disorders, uremic toxins, shortened red cell survival, blood loss on hemodialysis sessions or due to repeated blood sampling, lack of essential nutrients like folic acid and vitamin B12, inflammation are involved in CKD anemia. [6,7,8]

Considering the large number of possible etiological conditions, treatment of anemia is sometimes a difficult task for the practitioner.

Recent discovery of hepcidin, considered to be the most important regulator of circulating iron absorption was followed by research to prove her role in the etiology of anemia in chronic kidney disease. [9,10]

Moreover, serum hepcidin concentrations are influenced by inflammation and anemia of chronic kidney disease is often explained by the marked inflammatory status of these patients. [11,12]

In this setting, knowledge of the interplay between hepcidin, serum erythropoietin, inflammatory markers and glomerular filtration rate is a goal for nephrologists. Understanding the relationship between parameters would help us both to understand which categories of patients frequently develop anemia but also to answer an old question about different response to therapy of patients with chronic kidney disease anemia.

The purpose of our study was to compare hepcidin and inflammation markers in CKD patients with and without anemia and to assess the relationship with glomerular filtration rate, serum erythropoietin and inflammatory markers as fibrinogen, hsCRP, IL-6 and TNF-α.

Methods

The study was approved by local ethical board and was conducted according to the Declaration of Helsinki. All

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patients signed an informed consent for plasma sample collection and for the participation in the research.

Study population

Incident chronic kidney disease (CKD) patients referred for medical evaluation in the Nephrology Department of Mures County Clinical Hospital between March 2015 - June 2016 with the diagnosis of chronic kidney disease.

We considered eligible for the study two types of patients

1. Anemia group. Inclusion criteria: over 18 years, hemoglobin ≤12 g/dl for women and ≤13 g/dl for men, no treatment for anemia at least 6 months before enrolment, estimated glomerular filtration rate (eGFR) < 60 ml/min/1.73 mp and stable creatinine values three months before enrolment, ferritin < 300 ng/ml. Exclusion criteria: infection, bleeding, malignancy, systemic or liver disease, immunosuppression, MCV higher than 100 fl, renal function replacement therapy.
2. No anemia group. Inclusion criteria: over 18 years, hemoglobin >12 g/dl for women and >13 g/dl for men, no treatment for anemia at least 6 months before enrolment, estimated glomerular filtration rate (eGFR) < 60 ml/min/1.73 mp and stable creatinine values three months before enrolment. Exclusion criteria: medical conditions known to have a role in the development of polycythemia: chronic obstructive pulmonary disease, sleep apnea, morbid obesity, cardiovascular diseases with right-to-left shunt, renal carcinomas, adrenal carcinomas, hepatomas, adrenal adenomas.

Laboratory data

Blood samples were collected after an overnight fast, between 8:00 and 9:00 in the morning. Hemoglobin, hematocrit, serum creatinine was performed during routine examinations performed in the clinic.

For hepcidin, ferritin, erythropoietin and inflammatory markers assay, serum samples were stored at -80°C. Sample analysis was performed in the same session by the same examiner. Hepcidin-25 was measured by a immunoenzymatically colorimetric method (DRG® Hepcidin 25 bioactive ELISA, DRG International, Inc., USA) according to manufacturer´s indication, with an analytical sensitivity equal to 0.135 ng/ml.

For erythropoietin (EPO), tumor necrosis factor (TNF)-α, interleukin (IL)-6 assay we used a chemiluminescent enzyme immunometric assay (on a Siemens, Germany, Immulite1000 analyzer). Analytical sensitivity was 2 pg/ml for IL-6 assay, 1,7 pg/ml for TNF-alpha, 0.24 mIU/ml for EPO.

Ferritin concentrations were measured with an immunoenzymatically colorimetric method (ELISA Novatec Immundiagnostica GmbH, Dietzenbach, Germany), with an analytical sensitivity of 0.04 ng/ml.

Estimated glomerular filtration rate (eGFR) was calculated using 4-variable Modification of Diet in Renal Disease equation.

Statistical analysis

Statistical analysis was realized using GraphPad Prism 6 Software. Data were presented as mean ± standard deviation.

Differences between groups were evaluated with unpaired Student's t test.

Pearson test was used to verify the existence of correlations between parameters for data with Gaussian distribution and Spearman test was used for variables with non-Gaussian distribution.

Agostino- Pearson normality test was used to verify if values come from a Gaussian distribution. For regression models variables with non- normal distribution were logarithmically transformed.

Results

22 patients with chronic kidney disease anemia (14 females and 8 males) and 15 patients with CKD without anemia (6 females and 9 males) were investigated in the study.

In anemia group, hemoglobin values were significantly lower compared with patients without anemia. Mean age (standard deviation) was 59.55 ± 3.091 for the group with anemia and 62.87 ± 3.205 for the non-anemia group, with no significant differences between age groups. (Table 1)

There were no differences in terms of the mean glomerular filtration rate and body mass index between the two groups (Table 1).

We found no differences in serum hepcidin, erythropoietin, fibrinogen, IL-6, and TNF-α between CKD patients with and without anemia. Serum iron, TSAT, ferritin and (mean corpuscular volume) MCV showed no differences between the two groups. (Table I)

Table I. Clinical and laboratory parameters in patients with CKD

Parameter	Anemia group	Non-anemia group	P
eRFG (ml/min/1.73 mp)	23.75 ± 2.789	27.82 ± 2.882	NS
Hepcidin 25 (ng/ml)	30.48 ± 5.959	27.52 ± 4.405	NS
Body mass index (kg/mp)	27.93 ± 0.9404	30.15 ± 1.105	NS
Fibrinogen (mg/dl)	370.2 ± 28.25	377.7 ± 29.80	NS
Erythropoietin (mIU/mL)	11.12 ± 1.444	10.29 ± 1.084	NS
Age (years)	59.55 ± 3.091	62.87 ± 3.205	NS
TSAT (%)	21.88 ± 1.656	20.65 ± 2.183	NS
TNF-α (pg/mL)	11.45 ± 1.087	11.36 ± 1.101	NS
Ferritin (ng/mL)	86.07 ± 14.32	51.98 ± 9.612	NS
Il-6 (pg/mL)	5.14 ± 1.16	13.87 ± 7.95	NS
Hemoglobin (g/dl)	10.41 ± 0.267	13.84 ± 0.298	p<0.0001
MCV (fl)	85.93 ± 1.606	86.21 ± 1.411	NS
hsCRP (mg/L)	5.980 ± 1.063	8.920 ± 3.235	NS
Serum iron (µg/dL)	59.18 ± 5.125	59.50 ± 5.578	NS

Correlation analyses for all patients showed that serum hepcidin correlated positively with ferritin (r=0.45 p<0.05) and TNF-α (r=0.54, p<0.05). Hepcidin correlated negatively with erythropoietin (r=-0.51, p<0.05). We found no correlation between serum hepcidin and hemoglobin, iron, hsCRP, Il-6, fibrinogen and eGFR in our patients.

Multiple linear regression analysis for the whole population studied, with hepcidin as dependent and ferritin, erythropoietin and TNF-α as independent variables demonstrated that TNF-α is an independent predictor of serum hepcidin in our patients (p=0.003, R=0.71).

Discussion

Chronic kidney disease is a serious medical condition that evolves with numerous complications that reduces lifespan significantly. As filtration rate decreases, the prevalence of complications like cardiovascular disease, hyperparathyroidism or anemia increases significantly. [3] These big issues and spread in the population makes chronic kidney disease to be extensively studied in order to decrease associated comorbidities.

One important aspect of the disease is anemia. It is known that its prevalence increases with decreased glomerular filtration rate, charging the health care systems with huge costs. [13]

Chronic kidney disease anemia is a multifactorial process. Uremic milieu, low folate and B12 vitamin, malnutrition and inflammation are important causes of anemia. To these we can add frequent blood sampling in hospital setting or loss of blood in the hemodialysis circuit. Low erythropoietin production by the diseased kidney and disorders of iron metabolism are recognized as the main causes of anemia in chronic disease. [14,15]

Hepcidin is a relatively recently discovered molecule. The main function of hepcidin is to regulates iron absorption by binding to ferroportin, the only element involved in cellular iron export. [16]

The main site of production is the liver; hepcidin expression depends on iron stores but also by endogenous and exogenous EPO, infection, inflammation and hypoxia. [17]

Nephrologists community consider that hepcidin is an important issue because understanding its role in anemia of chronic kidney disease could provide important details on therapy.

This study focuses on characterizing of serum hepcidin profile in CKD patients with and without anemia and also on characterizing the relationship between hepcidin, glomerular filtration rate, endogenous EPO and inflammatory markers as fibrinogen, hsCRP, IL-6 and TNF-α.

We studied patients in whom history, clinical and paraclinical examination, has ruled out active infections or obvious causes of inflammation (see inclusion and exclusion criteria). Also, there were no significant differences in terms of age and glomerular filtration rate between the two groups of patients, to eliminate the possible impact of this parameters on serum hepcidin values.

Some authors demonstrated a negative correlation between serum hepcidin and glomerular filtration rate in CKD patients. [18,10]

Although this claim is not accepted by all nephrologists, in CKD serum hepcidin values are raised compared to the general population; it is also demonstrated a significant increase in hepcidin in hemodialysis patients. [19-22]

Previous studies demonstrated that patients with chronic kidney disease have increased plasma hepcidin levels and that patients with absolute iron deficiency have lower levels of hepcidin, the latter being considered a marker that can differentiate the anemia of chronic iron deficiency anemia. [23,24]

In our study, hemoglobin levels were different (p<0.0001) but hepcidin values were not different in anemia compared with non-anemia patients.

One possible explanation is lack of significant differences in terms of serum iron. Also, iron deficiency has not been a feature of the two groups studied and this may have influenced our results. Hepcidin expression is dictated by the body iron load [25]; and at comparable iron concentrations serum hepcidin values can also be comparable.

In these conditions, why some patients experience low hemoglobin values and others do not?

The question is even more present given that we demonstrated in the two groups of patients amounts of erythropoietin, IL-6 and TNF-α who do not show significant differences.

CKD anemia is a condition is a condition thought to be caused primarily by a lack of erythropoietin. Conversely, we would expect that patients without anemia present elevated serum erythropoietin.

Fehr et al found negative correlation between EPO and hemoglobin for a creatinine clearance above 40 mL / min. The author did not show a significant correlation below this clearance (despite a high rate of anemia) suggesting a lower set point for erythropoietin regulation. [26]

In a recent analysis of determinants of EPO in CKD, Mercadel et al postulated that CKD is characterized by early relative EPO deficiency, but several factors besides hemoglobin may persistently stimulate EPO synthesis ensuring normal hemoglobin levels in patients with measured GFR above 30 ml/min/ 1.73 mp. [27]

For both our groups mean glomerular filtration was under 30 ml/min/ 1.73 mp but with the specification that we used estimated and not measured glomerular filtration rate.

In our study, hepcidin correlated positively with ferritin. This correlation is the only one supported by all authors and it seems logical because the main regulator of hepcidin is the body's iron reserves. [28,29]

The fact that chronic kidney disease is a proinflammatory status that worsens as declining glomerular filtration is now universally accepted. [30,31]

Hepcidin production is directly regulated by proinflammatory cytokines. Nemeth et al demonstrated that hepci-

din is induced by IL-6 in human hepatocyte culture, and this response is rapidly followed by hypoferremia. [32]

Even if hepcidin expression is up-regulated by IL-6, CKD clinical studies are not consistent in supporting the correlation between hepcidin and IL-6. [33,34]

The negative correlation between EPO levels and hepcidin was an expected result; increased erythropoiesis requires a low hepcidin concentration that will allow absorption of iron needed to restore erythrocyte. [35]

Similar evidence was obtained recently regarding anemia in acute and chronic inflammatory processes where hepcidin- independent effects of inflammation on the suppression of erythropoiesis were demonstrated. [36]

In our study on non-dialysis chronic kidney disease, multiple regression analysis showed that TNF-α was the main predictor of hepcidin levels.

Inflammatory cytokines have active roles in influencing hepcidin expression. It is known that IL-6 has an important role in stimulating hepcidin but little are known about the direct influence of TNF-α.

Studies in rheumatoid arthritis patients showed that administration of TNF-α inhibitors reduced serum hepcidin-25 but the mechanisms are largely unknown. [37]

Our study limits are: limited number of patients, estimation of the glomerular filtration rate, that patients were not divided depending on the stage of chronic kidney disease.

In conclusion, our study has demonstrated lack of significant differences in terms of hepcidin profile and inflammatory markers in patients with CKD anemia compared to patients with CKD without anemia.

Hepcidin-25 does not correlate with glomerular filtration in non-dialysis chronic kidney disease and TNF-α was the main predictor of hepcidin-25 levels.

We conclude that hepcidin, iron markers, erythropoietin and inflammatory markers cannot fully explain chronic kidney disease anemia.

Chronic kidney disease anemia might be a more complex matter than we previously thought and further studies are necessary for provide the whole mechanism and to understand the role of hepcidin in iron metabolism in patients with CKD.

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Conflict of interest

None to declare.

References

1. Gilbertson D, Peng Y, Bradbury B, Ebben J, Collins A. Hemoglobin level variability: anemia management among variability groups. Am J Nephrol. 2009; 30: 491-498.
2. McClellan W, Aronoff S, Bolton W, Hood S, Lorber D, Tang K, et al. The prevalence of anemia in patients with chronic kidney disease. Curr Med Res Opin. 2004; 20: 1501-1510.
3. Astor B, Muntner P, Levin A, Eustace J, Coresh J. Association of kidney function with anemia: the Third National Health and Nutrition Examination Survey (1988-1994). Arch Intern Med. 2002; 162: 1401-1408.
4. Kohagura K, Tomiyama N, Kinjo K, Takishita S, Iseki K. Prevalence of anemia according to stage of chronic kidney disease in a large screening cohort of Japanese. Clin Exp Nephrol. 2009; 13: 614-620.
5. Stauffer E, Fan T. Prevalence of anemia in chronic kidney disease in the United States. PLoS ONE. 2014; 9: pe84943.
6. Eschbach J. The anemia of chronic renal failure: Pathophysiology and the effects of recombinant erythropoietin. Kidney Int. 1989; 35: 134-148.
7. Zadrazil J, Horak P. Pathophysiology of anemia in chronic kidney diseases: A review. biomed Pap Med Fac Univ Olomouc Czech Repub. 2015; 159: 197-202.
8. Babitt J, Lin H. Mechanisms of anemia in CKD. J Am Soc Nephrol. 2012; 23: 1631-1634.
9. Wagner M, Ashby D. Hepcidin—a well-known iron biomarker with prognostic implications in chronic kidney disease. Nephrol Dialysis Transplant. 2013; 28: 2936-2939.
10. Atkinson M, Kim J, Roy C, Warady B, White CT , Furth S. Hepcidin and risk for anemia in CKD: a cross-sectional and longitudinal analysis in the CKiD Cohort. Pediatr Nephrol. 2015; 30: 635-643.
11. Chonchol M, Lippi G, Montagnana M, Muggeo M, Targher G. Association of inflammation with anaemia in patients with chronic kidney disease not requiring chronic dialysis. Nephrol Dial Transplant. 2008; 23: 2879-2883.
12. Gupta J, Mitra N, Kanetsky P, Devaney J, Wing M, Reilly M, et al. Association between albuminuria, kidney function, and inflammatory biomarker profile in CKD in CRIC. Clin J Am Soc Nephrol. 2012; 12: 1938-1946.
13. Bruck K, Stel V, Gambaro G, Hallan S, Volzke H, Arnloy J, et al. CKD prevalence varies across the European general population. Am J Soc Nephrol. 2015; 27.
14. Chiang C, Tanaka T, Inagi R, Fujita T, Nangaku M. Indoxyl sulfate, a representative uremic toxin, suppresses erythropoietin production in a HIF-dependent manner. Lab Invest. 2011; 91: 1564-1571.
15. Al-Khoury S, Afzali B, Shah N, Thomas S, Gusbeth-Tatomir P, Goldsmith D, et al. Diabetes, kidney disease and anaemia: time to tackle a troublesome triad? Int J Clin Pract. 2007; 61: 281-289.
16. Ramey G, Deschemin J, Durel B, Canonne-Hergaux F, Nicolas G, Vaulont S. Hepcidin targets ferroportin for degradation in hepatocytes. Haematologica. 2010; 95: 510-504.
17. Tsuchiya K, Nitta K. Hepcidin is a potential regulator of iron status in chronic kidney disease. Therapeuric Apheresis and Dialysis. 2013; 17: 1-8.
18. Troutt J, Butterfield A, Konrad R. Hepcidin-25 concentrations are markedly increased in patients with chronic kidney disease and are inversely correlated with estimated glomerular filtration rates. J Clin Lab Analysis. 2013; 27: 504-510.
19. Weiss G, Theurl I, Eder S, Koppelstaetter C, Kurz K, Sonnweber T, et al. Serum hepcidin concentration in chronic haemodialysis patients: associations and effects of dialysis, iron and erythropoietin therapy. Eur J Clin Invest. 2009; 39: 883-890.
20. Malyszko , Malyszko J, Pawlak K, Mysliwiek M. Hepcidin, iron status and renal function in chronic renal failure, kidney transplantation and hemodialysis. Am. J. Hematol. 2006; 81: 832-837.
21. Uehata T, Tomosugi N, Shoji T, Sakaguchi Y, Suzuki A, Kaneko T, et al. Serum hepcidin-25 levels and anemia in non-dialysis chronic kidney disease patients: a cross-sectional study. Nephrol. Dial. Transplant. 2012; 27: 1076-1083.
22. Peters H, Laarakkers C, Swinkels D, Wetzels J. Serum hepcidin-25 levels in patients with chronic kidney disease are independent of glomerular filtration rate. Nephrol. Dial. Transplant. 2010; 25: 848-853.
23. Lukaszyk E, Lukaszyk M, Koc-Zorawska E, Tobolczyk J, Bodzenta-Lukaszyk A, Malyszko J. Iron status and inflammation in early stages of chronic kidney disease. Kidney Blood Press Res. 2015; 40: 366-373.
24. Mercadel L, Metzger M, Haymann J, Thervet E, Boffa J, Flamant M, et al. The relation of hepcidin to iron disorders, inflammation and hemoglobin in chronic kidney disease. PLos One. 2014; 9: e99781.
25. Young B, Zaritsky J. Hepcidin for clinicians. Clin J Am Soc Nephrol. 2009; 4: 1384-1387.
26. Fehr T, Ammann P, Garzoni D, Korte W, Fierz W, Rickji H, et al. Interpretation of erythropoietin levels in patients with various degrees of renal insufficiency and anemia. Kidney Int. 2004; 66: 1206-1211.

27. Mercadel L, Metzger M, Casadevall N, Haymann J, Karras A, Boffa J, et al. Timing and determinants of erythropoietin deficiency in chronic kidney disease. Clin J Am Soc Nephrol. 2012; 7: 35-42.
28. Camaschella C. Iron-deficiency anemia. N. Engl. J. Med. 2015; 372: 1832- 1843.
29. Ganz T, Nemeth E. Hepcidin and iron homeostasis. Biochim Biophys Acta. 2012; 1823: 1434-1443.
30. Rao M, Wong C, Kanetsky P, Girndt M, Sternvinkel P, Reilly M, et al. Cytokine gene polymorphism and progression of renal and cardiovascular diseases. Kidney Int. 2007; 72: 549-556.
31. Pecoits-Filho R, Lindholm B, Axelsson J, Stenvinkel P. Update on interleukin-6 and its role in chronic renal failure. Nephrol Dial Transplant. 2003; 18: 1042-1045.
32. Nemeth E, Rivera S, Gabayan V, Keller C, Taudorf S, Pedersen B, et al. IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. J Clin INvest. 2004; 113: 1271-1276.
33. Yang L, Zhang P, Tang F, Wang F, Li E. Correlation between hepcidin level and renal anemia. Genet Mol Res. 2014; 13: 7407-7410.
34. Jairam A, Das R, Aggarwal P, Kohli H, Gupta K, Sakhuja V, et al. Iron status, inflammation and hepcidin in ESRD patients: The confounding role of intravenous iron therapy. Indian Journal of Nephrology. 2010; 20: 125-131.
35. Gammella E, Diaz V, Recalcati S, Buratti P, Samaja M, Dey S, et al. Erythropoietin's inhibiting impact on hepcidin expression occurs indirectly. Am J Physiol Regul Integr Comp Physiol. 2015; 308: R330-R335.
36. Langdon J, Yates S, Femnou L, McCranor B, Cheadle C. Hepcidin-dependent and hepcidin-independent regulation of erythropoiesis in a mouse model of anemia of chronic inflammation. Am J Hematol. 2014: 470-479.
37. Song S, Iwahashi M, Tomosugi N, Uno K, Yamana J, Yamana S, et al. Comparative evaluation of the effects of treatment with tocilizumab and TNF-α inhibitors on serum hepcidin, anemia response and disease activity in rheumatoid arthritis patients. Arthritis Res Ther. 2013; 15: R141.