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Impact of physiological and functional disorders of the kidneys on iron metabolism

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In chronic kidney disease (CKD), functional impairments of the kidneys can lead to disturbances in iron metabolism, resulting in iron deficiency and the development of anemia. This study aimed to investigate the impact of physiological and functional renal impairments on iron metabolism and to explore the molecular mechanisms involved in this process. The study included 44 patients with stage II–III CKD who were not undergoing hemodialysis (conservative group), and 46 patients with end-stage CKD receiving regular hemodialysis (terminal group). Blood samples from all participants were analyzed to determine the concentrations of iron, creatinine, urea, erythropoietin, hepcidin, ferroportin, lactoferrin, and haptoglobin. Functional impairment of the kidneys during CKD leads to significant changes in iron metabolism, depending on the stage of disease progression. Specifically, patients with CKD showed a significant decrease in serum levels of iron, erythropoietin, and haptoglobin, while levels of hepcidin, ferroportin, and lactoferrin were markedly increased. In the terminal stage of the disease, disturbances in iron metabolism were accompanied by more severe anemia. Thus, the pathogenesis of anemia in CKD is not solely due to erythropoietin deficiency but is also shaped by complex molecular and cellular mechanisms involving iron metabolism proteins. Functional deterioration of the kidneys results in significant alterations in iron metabolism, which correlate with disease progression. Among the proteins involved, hepcidin, ferroportin, and lactoferrin are considered specific and sensitive markers for determining the etiology of CKD-associated anemia.

Keywords: *Chronic kidney disease, erythropoietin, hepcidin, ferroportin, lactoferrin, haptoglobin*

INTRODUCTION

Relevance of the research: The kidneys are among the main organs responsible for maintaining homeostasis. Physiological and functional disorders affecting them can significantly impact many systems, including iron metabolism. The kidneys are not limited to filtration and the maintenance of homeostasis. They also play a crucial role in iron reabsorption and the regulation of its balance. Iron metabolism is vital for oxygen transport, cellular respiration, and enzymatic processes in the body. In particular, during chronic kidney disease (CKD), functional impairment of the kidneys can lead to disturbances in iron metabolism, ultimately resulting in iron deficiency and the development of anemia (Murkamilov and Fomin, 2017).

Impaired iron absorption and both functional and absolute iron deficiency due to chronic inflammation are key factors contributing to the suppression of erythropoiesis. In CKD, the accumulation of uremic toxins, such as indoxyl sulfate interferes with iron metabolism, thereby exacerbating anemia. These toxins affect the synthesis of erythropoietin (EPO) in the kidneys, leading to renal hypoxia and playing a significant role in the pathophysiology of renal anemia (Koury and Haase, 2015). According to WHO data, in 90% of CKD patients with a glomerular

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filtration rate (GFR) of 25 mL/min, hemoglobin levels fall below 10 g/dL (Yakupova et al., 2023).

In CKD, the systemic inflammatory state increases the synthesis of hepcidin in hepatocytes. Elevated levels of hepcidin lead to the degradation of ferroportin, thereby restricting the release of iron from storage sites, such as the liver, macrophages, and kidney cells, into the circulation. Ferroportin is primarily located in the proximal tubule cells of the kidneys and facilitates the return of reabsorbed iron into the systemic circulation. Therefore, increased hepcidin synthesis results in functional iron deficiency and the development of normocytic normochromic anemia (Ganz and Nemeth, 2012; Wojtaszek et al., 2020; Jonny et al., 2023).

In addition to erythropoietin, hepcidin, and ferroportin, the proteins haptoglobin and lactoferrin also play important roles in iron metabolism.

Haptoglobin (Hp) is synthesized in the liver and binds to free hemoglobin released during hemolysis, facilitating its transport to the reticuloendothelial system, particularly to macrophages in the liver and spleen. This process not only enables the recycling of iron but also prevents oxidative damage that could be caused by free hemoglobin (Schaer et al., 2013). The haptoglobin-hemoglobin complex enters monocytes and macrophages via endocytosis mediated by the CD163 receptor. As a result, heme-bound iron is absorbed by macrophages and is either stored in the form of ferritin and hemosiderin or transported to other tissues via transferrin (Huang et al., 2019).

Lactoferrin is a naturally occurring iron-binding glycoprotein synthesized by the epithelial cells of mucous membranes. It possesses antiviral, antibacterial, and antioxidant properties. Due to its antioxidant activity, lactoferrin plays a protective role in protecting the kidneys against damage (Hsu, Chiu et al. 2020).

Recent scientific studies have shown that the relationship between impaired kidney function and iron metabolism requires a comprehensive approach. In treatment, it is not sufficient to administer iron alone. It is also important to regulate hepcidin levels and use erythropoietin analogs.

The role of iron metabolism proteins in the pathogenesis of anemia in CKD is still not fully understood. Investigating the molecular mechanisms of iron metabolism in CKD may play a crucial role in the prevention and treatment of anemia in these patients.

The study aimed to investigate the impact of physiological and functional disorders of the kidneys on iron metabolism, as well as to explore the molecular mechanisms involved in this process.

Object: The study was conducted with 44 patients of stage II–III CKD who were not receiving hemodialysis and with the terminal group, consisting of 46 patients with terminal stage of CKD who were undergoing regular hemodialysis.

MATERIALS AND METHODS

In this study, blood samples were analyzed from two groups of patients: the conservative group, consisting of 44 patients aged between 43 and 75 years (66.2±1.7 years on average) with stage II–III CKD who were not receiving hemodialysis and the terminal group, consisting of 46 patients aged between 19 and 77 years (60.8±2.2 years on average) with terminal stage of CKD who were undergoing regular hemodialysis. In the conservative group, there were 17 female patients (68.9±3.0 years) and 27 male patients (64.5±2.0 years). Among patients with terminal stage CKD (tCKD), 24 were women (62.4±2.8 years) and 22 were men (59.0±3.4 years). The control group included 20 practically healthy individuals aged between 20 and 75 years (62.5±1.9 years on average). To assess the severity of CKD, the concentrations of iron, creatinine, and urea in the patients' blood were measured. The concentrations of iron, creatinine, and urea in blood serum were determined by a spectrophotometric method using reagent kits from the company "Human." The concentrations of erythropoietin, hepcidin, ferroportin, haptoglobin, and lactoferrin in blood serum were analyzed by immunoenzyme method using reagent kits provided by BT LAB Bioassay Technology Laboratory (Shanghai, China). The informativeness (specificity and sensitivity) of the iron metabolism proteins was assessed by the ROC statistical analysis method.

Statistical analysis: The statistical analysis of the data was performed using a non-parametric method with the Wilcoxon–Mann–Whitney test.

RESULTS AND DISCUSSION

In the conservative group, the median concentrations of creatinine and urea in men, increased by 2.3 times ($p<0.001$) and 2.5 times ($p<0.001$), respectively. In women, these increases were 2.0 times ($p<0.001$) and 2.9 times ($p<0.001$) compared to the control group. In the terminal group undergoing hemodialysis, the median concentrations of creatinine and urea were found to be increased by 7.6 times ($p<0.001$) and 7.4 times ($p<0.001$) in men, and by 8.2 times ($p<0.001$) and 8.4 times ($p<0.001$) in women compared to the control group (Table 1).

The progression of CKD in patients was accompanied by functional iron deficiency and anemia. According to the results, in the conservative group, the concentrations of hemoglobin and iron decreased by 19% (1.2-fold; $p=0.010$) and 67% (1.7-fold; $p<0.001$) in men, and by 17% (1.2-fold; $p=0.008$) and 44% (1.4-fold; $p<0.001$) in women compared to the control group. In the terminal group, statistically significant decreases in hemoglobin and iron concentrations were observed, with reductions of 53% (1.5-fold; $p<0.001$) and 2.0-fold ($p<0.001$) in men, and 64% (1.6-fold; $p<0.001$) and 2.2-fold ($p<0.001$) in women compared to controls (Tables 2 and 3).

During CKD, a decrease in erythropoietin concentration depending on the stage of the disease was also observed. The concentration of erythropoietin decreased compared to the control group: in the conservative group, by 2.1 times in men ($p<0.001$) and by 58% (1.6 times; $p<0.001$) in women; in the terminal group, by 2.9 times in men ($p<0.001$) and by 2.3 times in women ($p<0.001$), which was not statistically significant.

Table 1. Indicators of kidney functional status in CKD patients

Indicators	Groups												P
	Control				Conservative				Terminal				
	Males												
	M	Me	Q1	Q3	M	Me	Q1	Q3	M	Me	Q1	Q3	
Creatinine, mol/L	81.6	81.0	71.0	94.0	197.3	181.0	123.0	238.0	674.8	618.5	472.0	789.0	<0.001
Urea, µmol/L	4.5	4.6	3.6	5.0	14.1	11.3	9.6	16.3	42.0	33.9	18.9	48.5	<0.001
	Females												
Creatinine, mol/L	73.6	71.0	61.0	89.0	193.6	138.8	110.5	251.9	624.7	585.0	506.0	799.5	<0.001
Urea, µmol/L	4.0	4.1	3.4	4.4	13.2	11.8	8.9	14.2	33.5	34.4	23.3	42.8	<0.001

Note: p -compared to the control

Table 2. Concentration of iron metabolism proteins in male patients with CKD

Indicators	Groups (males)											
	Control				Conservative				Terminal			
	M	Me	Q1	Q3	M	Me	Q1	Q3	M	Me	Q1	Q3
Erythropoietin, mIU/mL	2167.0	2320.0	2010.0	2404.0	1113.3	1089.0	935.0	1254.0	728.4	797.0	491.0	871.0
p							<0.001				<0.001	
Lactoferrin, ng/mL	0.934	0.970	0.720	1.090	1.186	1.160	1.020	1.340	2.605	2.500	2.000	3.100
p							0.014				<0.001	
Iron, µmol/L	19.9	20.0	17.0	23.0	12.0	12.0	10.4	13.9	9.7	9.8	8.5	12.3
p							<0.001				<0.001	
Haptoglobin, mol/L												
p							0.021				0.001	
Ferroportin, ng/mL	0.31	0.30	0.27	0.35	0.58	0.56	0.49	0.66	0.95	0.84	0.72	1.13
p							<0.001				<0.001	
Hepcidin, ng/ml	175.5	165.0	159.0	204.0	191.2	197.0	149.0	240.0	402.5	359.0	317.0	444.0
p							0.260				<0.001	

Note: p - compared to the control

Table 3. Concentration of iron metabolism proteins in female patients with CKD

Indicators	Groups (females)											
	Control				Conservative				Terminal			
	M	Me	Q1	Q3	M	Me	Q1	Q3	M	Me	Q1	Q3
Erythropoietin, mIU/mL	2003.9	1925.0	1889.0	2072.0	1185.7	1217.0	986.0	1340.0	798.8	821.0	715.5	940.5
p						<0.001				<0.001		
Lactoferrin, ng/mL	0.963	0.960	0.780	1.200	1.462	1.440	1.320	1.660	2.621	2.700	2.100	3.150
p						0.002				<0.001		
Iron, µmol/L	14.8	14.0	13.0	16.0	9.9	9.7	8.7	10.5	6.6	6.5	5.6	8.0
p							<0.001				<0.001	
Haptoglobin, mol/L	255.4	253.0	243.0	263.0	194.4	213.0	160.0	230.0	156.5	147.5	134.0	184.0
p							0.001				<0,001	
Ferroportin, ng/mL	0.36	0.36	0.34	0.38	0.53	0.52	0.38	0.63	0.86	0.76	0.68	0.93
p							0,004				<0.001	
Hepcidin, ng/ml	164.8	165.0	157.0	176.0	166.3	179.0	137.0	204.0	306.0	295.5	258.5	330.5
p							0.359				<0.001	

Note: p -compared to the control

In these patients, a significant increase in the concentrations of proteins involved in iron metabolism was also observed. Although the median concentration of hepcidin in the conservative group tended to increase compared to the control group, by 19% in men (p=0.260) and 8% in women (p=0.359), in the terminal stage, the hepcidin concentration increased by 2.2 times in men (p<0.001) and 1.8 times in women (p<0.001), which was statistically significant.

An increase in ferroportin concentration was also observed in patients with CKD. In the conservative group, the median concentration of ferroportin was significantly higher compared to the control group - by 87% (1.9 times; p<0.001) in men and by 44% (1.4 times; p=0.004) in women. In the terminal group, the increase was even more pronounced – 2.8 times in men (p<0.001) and 2.1 times in women (p<0.001).

The concentration of lactoferrin, one of the key proteins involved in iron metabolism, in the conservative group, increased by 20% (1.2 times; p=0.014) in men and by 50% (1.5 times; p=0.002) in women compared to the control group. In the terminal group, the increase was 2.6 times in men (p<0.001) and 2.8 times in women (p<0.001), which was statistically significant.

The ROC statistical method was used to evaluate the informativeness of iron metabolism proteins in the etiology of CKD-related anemia. The obtained results are presented in the graph illustrating the ROC curve and its parameters (Graph).

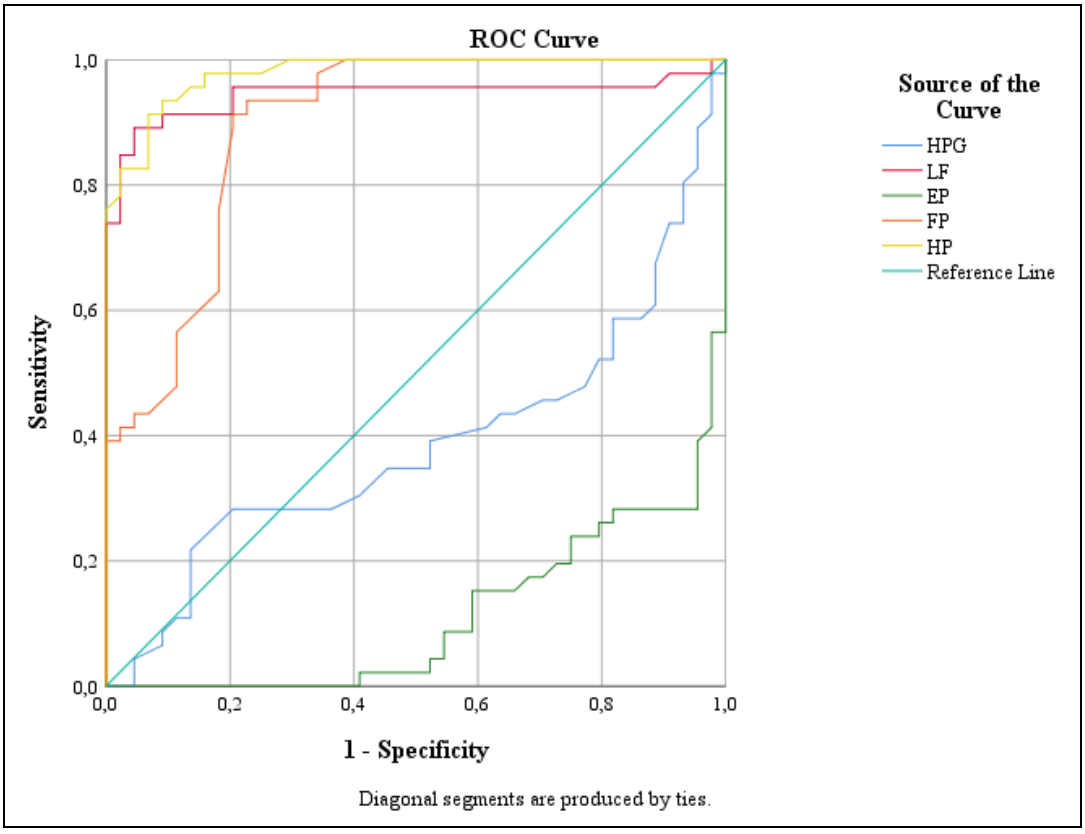
According to ROC statistical analysis, the level of lactoferrin (AUC=0.944, 95% CI: 0.887–1.000; p<0.001) can be considered a highly specific and sensitive marker in the etiology of anemia in CKD patients. As shown in the ROC curves, the levels of ferroportin (AUC=0.894, 95% CI: 0.828–0.960; p<0.001) and hepcidin (AUC=0.979, 95% CI: 0.957–1.000; p<0.001) are also specific and sensitive indicators for predicting anemia.

According to the ROC statistical analysis, haptoglobin (AUC=0.387, 95% CI: 0.267–0.506; p=0.064) and erythropoietin (AUC=0.111, 95% CI: 0.047–0.176; p<0.001) cannot be considered specific or sensitive markers in the etiology of anemia in CKD patients.

As the results indicate, functional impairments of the kidneys during CKD have a significant impact on iron metabolism. In patients with CKD, the progression of the inflammatory process caused by damage to the renal tubules and glomeruli leads to an increase in pro-inflammatory cytokines, which in turn disrupts iron homeostasis (Koury and Haase, 2015). Structural and morphological changes occurring in the kidneys affect not only iron metabolism but also the synthesis of the hormone erythropoietin. It is well known that the kidneys are the main site of erythropoietin production. Erythropoietin stimulates the production of red blood cells, and the acceleration of erythropoiesis increases the body's demand for iron. EPO also promotes iron utilization by enhancing the synthesis of

transferrin receptors. During CKD, degenerative and dystrophic changes in the renal parenchyma significantly reduce the synthesis of erythropoietin, leading to impaired erythropoiesis and an imbalance in iron consumption. The reduction in renal EPO synthesis results in a marked decrease in erythrocyte production, constituting one of the main mechanisms of CKD-associated anemia (Babitt et al., 2012). The decreased utilization of iron in erythropoiesis leads to a reduction in transferrin synthesis (Koury and Haase, 2015).

During CKD, the activation of inflammatory processes and the increased levels of pro-inflammatory cytokines lead to accelerated synthesis of hepcidin. Inflammatory cytokines, particularly IL-6, directly stimulate the transcription of hepcidin. Hepcidin is a liver-derived peptide hormone that plays a key role in the homeostatic regulation of iron in the body. It binds to ferroportin, an iron-exporting protein located on intestinal enterocytes and macrophages of the reticuloendothelial system, leading to its internalization and degradation.



Area Under the Curve

Test Result Variable(s)	Area	Std. Error	Asymptotic Sig.	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
Haptoglobin	0.387	0.061	0.064	0.267	0.506
Lactoferrin	0.944	0.029	0.000	0.887	1.000
Erythropoietin	0.111	0.033	0.000	0.047	0.176
Ferroportin	0.894	0.034	0.000	0.828	0.960
Hepcidin	0.979	0.011	0.000	0.957	1.000

Graph. Informativeness of iron metabolism proteins in patients with CKD.

As a result, elevated hepcidin levels limit both the absorption of dietary iron in the intestines and the release of stored iron from the reticuloendothelial system. This leads to

"functional iron deficiency" - a condition in which iron is present in the body but is not accessible for hematopoiesis (Ganz and Nemeth, 2012; Mohammad et al., 2021). Iron blockade due to increased hepcidin reduces sensitivity to erythropoietin, as the differentiation of erythroblasts into erythrocytes is an iron-dependent process. Maintaining iron balance is more difficult in patients undergoing hemodialysis or peritoneal dialysis. During hemodialysis, a portion of iron is lost from the blood. At the same time, due to the frequent occurrence of inflammatory conditions in these patients, hepcidin levels increase, which reduces iron utilization. Since oral iron supplements are ineffective in this group, there is a need for parenteral (intravenous) iron preparations. Hepcidin is degraded in the kidneys and excreted in the urine. Therefore, one of the reasons for elevated hepcidin levels is impaired renal clearance (Hain et al., 2023). According to the literature data, the administration of epoetin alfa and other erythropoietin analogs as a primary treatment for patients with CKD also contributes to functional iron deficiency. This is because, after each dose of the drug, the rate of erythropoiesis temporarily reaches its maximum. However, the release of iron from storage sites is not rapid enough to support this accelerated erythropoiesis, leading to a relative deficiency of circulating iron (Hain et al., 2023).

Another factor that further limits iron availability is the protein lactoferrin (Kell et al., 2020). Lactoferrin is stored in the specific granules of neutrophils and is released into plasma and body fluids during inflammation. Its levels increase particularly under conditions of chronic inflammation, including CKD, as this protein is involved in the regulation of inflammatory mediators. Lactoferrin improves iron utilization and stimulates erythropoiesis by reducing the levels of inflammatory markers such as interleukin-6 (IL-6) and hepcidin. This highlights the additional benefits of lactoferrin in the treatment of anemia in patients with CKD and those undergoing hemodialysis (González-Chávez et al., 2009). During hemodialysis, exposure to endotoxins, activation of the immune system, and oxidative stress further increase lactoferrin secretion. This also reflects the activation of its antimicrobial and immunomodulatory properties (Abad et al., 2025). During CKD, the increase in lactoferrin concentration occurs as a compensatory response to the reduction of serum iron levels and the progression of the inflammatory process (Cutone et al. 2019; Hain et al., 2023).

On the other hand, during CKD, oxidative stress and the effects of uremic toxins shorten the lifespan of red blood cells and increase the rate of hemolysis. Under this condition, the free hemoglobin released into the plasma is bound by a protein called haptoglobin, and this complex is transported to the liver for utilization. However, since the level of haptoglobin is reduced in CKD, the neutralization of free hemoglobin is impaired, leading to additional heme toxicity and ineffective iron utilization (Schaer et al., 2013). Recent proteomic studies have shown that the levels of haptoglobin are elevated in the urine and blood of patients with CKD. This indicates the potential role of haptoglobin as a biomarker for CKD and reveals a negative correlation with renal GFR. Thus, in addition to preventing the filtration and renal excretion of hemoglobin and mitigating oxidative stress, Hp ensures the effective and safe circulation of iron in the body. Due to these properties, haptoglobin plays an important role in protecting kidney tissue from damage (Huang et al., 2019; Wang et al., 2019).

CONCLUSION

Thus, in CKD, the pathogenesis of anemia is not solely attributed to erythropoietin deficiency but also involves complex molecular and cellular mechanisms related to iron metabolism proteins. Due to impaired renal function in CKD, significant alterations occur in iron metabolism depending on the stage of disease progression. In CKD patients, the levels of erythropoietin and haptoglobin markedly decreased while on the contrary, the levels of hepcidin, ferroportin, and lactoferrin increased. In the terminal stage of the disease, disturbances in iron metabolism are accompanied by more severe anemia. Among the proteins involved, hepcidin, ferroportin, and lactoferrin are considered specific and sensitive markers in identifying the etiology of CKD-

related anemia. A more comprehensive understanding of the relationship between impaired renal function and iron metabolism in CKD, as well as its consideration in clinical practice, may contribute to the effective treatment of renal anemia and lead to better quality of life for patients.

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