Original paper
Originalni naučni rad

INTERPRETATION OF ERYTHROPOIETIN AND HAEMOGLOBIN LEVELS IN PATIENTS WITH VARIOUS STAGES OF CHRONIC KIDNEY DISEASE

TUMAČENJE NIVOA ERITROPOJETINA I HEMOGLOBINA KOD PACIJENATA U RAZLIČITIM FAZAMA HRONIČNE BUBREŽNE INSUFICIJENCIJE

Mirsad Panjeta[1,2], Ismet Tahirović[1], Emin Sofić[1,3], Jozo Ćorić[2], Amela Dervišević[4]

[1]University of Sarajevo, Faculty of Science, Department of Chemistry, Bosnia and Herzegovina
[2]Clinical Center University of Sarajevo, Institute for Clinical Chemistry and Biochemistry, Bosnia and Herzegovina
[3]Faculty of Pharmacy, University of Sarajevo, Bosnia and Herzegovina
[4]Department of Human Physiology, Faculty of Medicine, University of Sarajevo, Bosnia and Herzegovina

Summary

Background: The production of erythrocytes is regulated by the hormone erythropoietin (EPO), which maintains the blood haemoglobin (Hb) levels constant under normal conditions. Human EPO is a glycoprotein hormone and its synthesis is controlled by the hypoxia-inducible transcription factor. The aim of this study was to establish EPO and Hb levels in patients with chronic kidney disease (CKD), as well as in control subjects, and to investigate the relationship between these parameters.
Methods: This cross-sectional, observational study included 356 subjects with CKD divided into 4 subgroups according to their glomerular filtration rate (GFR). The control group consisted of 206 age and sex matched healthy subjects with GFR rate ≥90 mL/min/1.73 m^2. EPO, Hb and serum creatinine levels were determined by using immunochemical and spectrophotometric methods. GFR was determined using the MDRD formula.
Results: The CKD patients had significantly lower levels of haemoglobin (p<0.0005) and hematocrit (p<0.0005) compared to control group. Our results showed that Hb levels decreased, whereas serum creatinine increased with the increasing renal failure. The CKD patients in all four groups had significantly lower (p<0.0005) Hb levels, and significantly higher (p<0.0005) creatinine levels compared to the control group. The median EPO in group I and II were significantly higher (p=0.002; p=0.018), while median EPO in

Kratak sadržaj

Uvod: Proizvodnju eritrocita reguliše hormon eritropojetin (EPO), koji u normalnim uslovima održava konstantne nivoe hemoglobina (Hb) u krvi. Humani EPO je glikoproteinski hormon i njegovu sintezu kontroliše hipoksija-inducibilni transkripcioni faktor. Cilj ove studije bio je da se ustanove nivoi EPO i Hb kod pacijenata sa hroničnom bubrežnom insuficijencijom (HBI) kao i kod kontrolnih subjekata, te da se istraži odnos između ovih parametara.
Metode: Ova opservaciona studija preseka obuhvatila je 356 subjekata sa HBI podeljenih na 4 podgrupe prema stopi glomerulske filtracije (GFR). Kontrolnu grupu činilo je 206 zdravih osoba odgovarajuće starosti i pola sa stopom GFR ≥90 mL/min/1,73 m^2. EPO, Hb i nivoi kreatinina u serumu određeni su imunohemijskim i spektrofotometrijskim metodama. GFR je određena pomoću formule MDRD.
Rezultati: Oboleli od HBI imali su značajno niže nivoe hemoglobina (p<0,0005) i hematokrita (p<0,0005) u poređenju sa kontrolnom grupom. Naši rezultati pokazuju da se nivoi Hb smanjuju, dok kreatinin u serumu raste s povećanjem stepena bubrežnog oštećenja. Oboleli od HBI u sve četiri grupe imali su značajno niže (p<0,0005) nivoe Hb i značajno više (p<0,0005) nivoe kreatinina u poređenju sa kontrolnom grupom. Srednji nivo EPO-a u grupi I i II bio je značajno viši (p=0,002; p=0,018) dok je srednji nivo EPO-a u grupi III i IV bio značajno niži (p=0,03; p=0,011) u poređenju sa kontrolnom grupom.

Address for correspondence:

Ismet Tahirović
Department of Chemistry, Faculty of Science,
University of Sarajevo
Phone: ++ 387 33 279 905; Fax: ++ 387 33 649-359
e-mail: itah@pmf.unsa.ba

group III and IV were significantly lower (p=0.03; p=0.011) compared to the control group.

Conclusions: In patients with CKD, GFR positively correlated with Hb and EPO, while the correlation between GFR and serum creatinine was negative.

Keywords: erythropoietin, haemoglobin, chronic kidney disease

Zaključak: Kod pacijenata sa HBI, GFR je bila u pozitivnoj korelaciji sa Hb i EPO-om, dok je korelacija između GFR i kreatinina u serumu bila negativna.

Ključne reči: eritropojetin, hemoglobin, hronična bubrežna insuficijencija

Introduction

The main humoral regulator of erythropoiesis is the hormone erythropoietin (EPO) (1, 2). EPO is a glycoprotein hormone composed of 165 amino acid residues with four complex carbohydrate chains attached to peptide in four binding positions. The kidneys are the primary source of EPO and its synthesis is controlled by hypoxia-inducible transcription factors (HIFs) (3). HIF is activated in all cells by exposure to hypoxia, as well as cobalt or iron chelators (4). However, under low-oxygen conditions (hypoxia), the number of circulating red blood cells increases to deliver oxygen efficiently into peripheral organs. Therefore, under hypoxic conditions, the kidney will produce and secrete EPO to increase the proliferation of erythrocytes by targeting the colony-forming unit-erythroid (CFU-E), proerythroblast and basophilic erythroblast subsets in the differentiation (2, 5). EPO binds to its receptor (EpoR) on the surfaces of immature erythroid cells and stimulates signalling cascades for proliferation, differentiation, and antiapoptosis (6).

The plasma concentration of EPO depends on the rate of its secretion in the kidney, and is inversely related to the oxygen content of the blood (2, 3). The dynamics of the secretion of EPO into circulation and, hence, its plasma concentrations are mainly determined by the transcription rate of the EPO gene, which itself is essentially under control of the cellular oxygen content. A decrease in oxygen concentrations increases the activity of the hypoxia-inducible transcription factor (HIF), which in turn triggers EPO gene transcription. Disorders of kidney function lead to abnormal EPO secretion, which may result in severe anaemia or polycythemia (7, 8). In patients with chronic kidney disease (CKD), normochromic normocytic anaemia may develop due to EPO deficiency (7–9). Although anaemia in patients with CKD is multifactorial in origin, it is primarily associated with erythropoietin production deficiency as the glomerular filtration rate (GFR) falls. Once the estimated GFR trends below 60 mL/min/1.73 m^2 (Stage 3a CKD), erythropoietin production by the kidneys falls, and anaemia may develop (10). Many studies have shown that every tenth person in the world has a CKD. Renal anaemia is a consequence of CKD and occurs at an early stage, and it gets worse as the disease progresses (11, 12).

According to the WHO, anaemia is defined as the haemoglobin concentration less than 130 g/L for men and less than 120 g/L for premenopausal women (13). Anaemia is a frequent complication of CKD, occurring in over 90% of patients receiving renal replacement therapy and is associated with high risk of death. However, the process is multifactorial, with several other contributing factors: absolute and functional iron deficiency, folate and vitamin B$_{12}$ deficiencies, reduced red blood cell life span, and suppression of erythropoiesis (14).

Apart from the mentioned facts, anaemia in kidney patients is not recognized and treated at the right time; therefore, the aim of this study was to evaluate EPO and Hb levels in patients with CKD and in control subjects, and to investigate the relationship between these parameters.

Materials and Methods

This cross-sectional, observational study included 562 subjects: a group of 356 patients with CKD (174 males and 182 females; age 61.7±16.1 years) and a control group consisting of 206 healthy subjects (104 males and 102 females; age 59.2±14.4 years).

CKD was defined in accordance with the Kidney Disease Initiatives and Global Outcome guidelines (15). The subjects with CKD were classified into four CKD stages according to GFR rate: Group I (n=88): patients with stage 1 CKD – GFR = 60.00–89.99 mL/min/1.73 m^2, Group II (n=141): patients with stage 2 CKD – GFR = 30.00–59.99 mL/min/1.73 m^2, Group III (n=73): patients with stage 3 CKD – GFR = 15.00–29.99 mL/min/1.73 m^2, and Group IV (n=54): patients with stage 4 CKD – GFR ≤ 14.99 mL/min/1.73 m^2.

The control group included apparently healthy subjects with GFR rate ≥90 mL/min/1.73 m^2, who did not suffer from any form of anaemia, liver or renal disease, or had any bone marrow complications. The Modification of Diet in Renal Disease (MDRD) formula was used for the calculation of GFR (16). All CKD patients underwent a standard diagnostic protocol comprising a detailed anamnestic questionnaire, medical history and physical examination. Blood samples from CKD patients and healthy subjects were obtained in the morning after a 12-h fasting period. The blood samples were collected into empty tubes

and immediately stored at +4 °C. Biochemical analyses were performed on the same day.

Erythropoietin levels were determined using the enzymatic-chemiluminescent immunometric method on IMMULITE/IMMULITE 1000 systems from Siemens Healthcare Diagnostics. Haemoglobin concentration was determined by the counter of blood elements (Cell Dyn 3700 system), using a haemoglobin-hydroxylamine colorimetric method. Creatinine was determined by a modified kinetic Jaffe's reaction reported by Larsen (17), on an autoanalyzer Dimension RxL Siemens.

The study protocol was performed in accordance with the Helsinki Declaration as revised in 2000. Upon careful explanation of the study procedure, all subjects signed written consent for participation in the study. The study protocol was approved by the Institute for Research and Development of the Clinical Center University of Sarajevo (Approval number: 0901-2-50/15).

Statistical analysis

Data distribution was determined by the Kolmogorov-Smirnov test for normality. Data was reported as median and interquartile range (IQR). Variable dif-

ferences between the groups were determined by the nonparametric Mann-Whitney U-test. Correlation coefficient between variables was assessed by Spearman's test. Statistical significance was set at $p < 0.05$. For statistical analysis of the obtained data, we used the software package SPSS for Windows (version 19.0, SPSS Inc., Chicago, Illinois, USA).

Results

Median haemoglobin concentrations were significantly lower ($p < 0.0005$) in Group I [136.5 g/L (128.3–147.8 g/L)], Group II [127.0 g/L (111.5–140.0 g/L)], Group III [114.0 g/L (105.0–128.5 g/L)] and Group IV [115.0 g/L (98.0–123.0 g/L)] than in the control group [146.0 g/L (140.0–152.0 g/L)]. Median haemoglobin concentration in Group I was statistically higher ($p < 0.0005$) than in Group II, III, and IV. A significant difference in haemoglobin concentration was also found when Group II was compared to Group III ($p = 0.002$) and Group IV ($p < 0.0005$). Comparison of haemoglobin concentrations between other groups showed no significant differences (*Figure 1*).

Patients in all stages of CKD had significantly higher median serum creatinine concentrations than
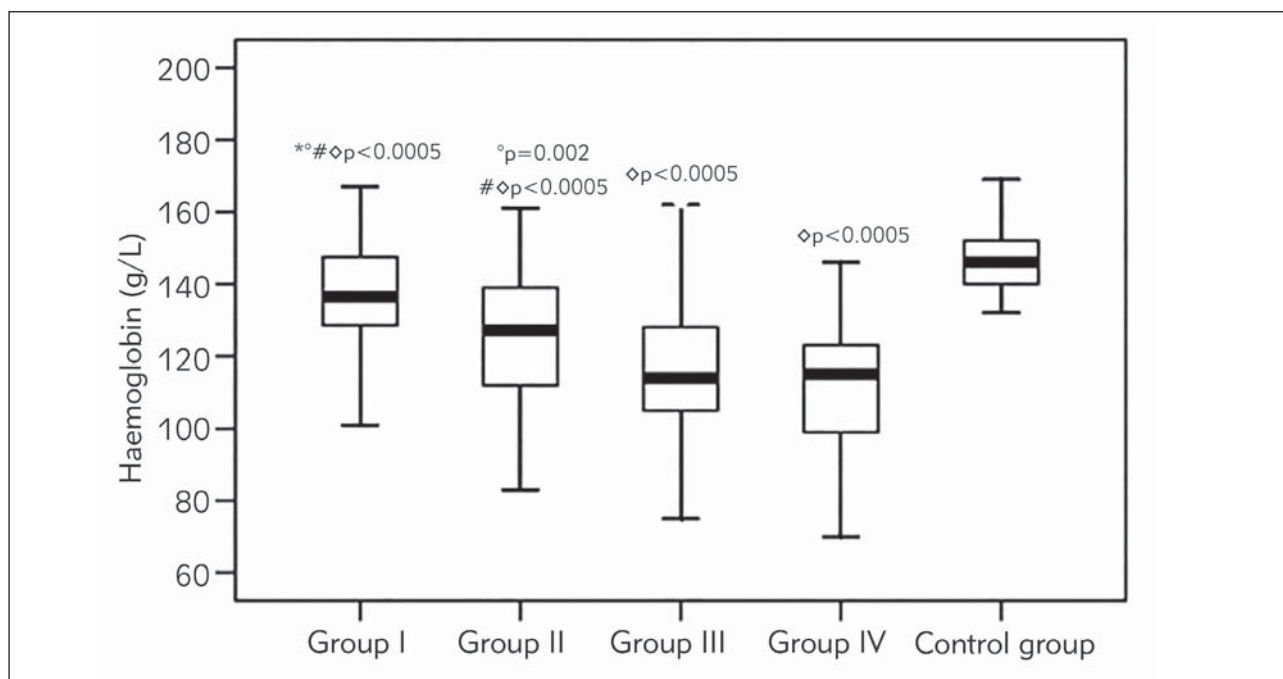

Figure 1 Box-and-whisker plots of haemoglobin concentration (g/L) in the studied groups.
The solid horizontal lines denote the median value, the box represents 25% and 75% interquartile ranges and the whiskers represent minimum and maximum values.
Group I – patients with stage 1 CKD (GFR=60.00–89.99 mL/min/1.73 m²); Group II – patients with stage 2 CKD (GFR=30.00–59.99 mL/min/1.73 m²); Group III – patients with stage 3 CKD (GFR=15.00–29.99 mL/min/1.73 m²); Group IV – patients with stage 4 CKD (GFR ≤14.99 mL/ min/1.73 m²)
p – probability; NS – not significant; * – compared to Group II; ° – compared to Group III; # – compared to Group IV; ◊ – compared to Control group
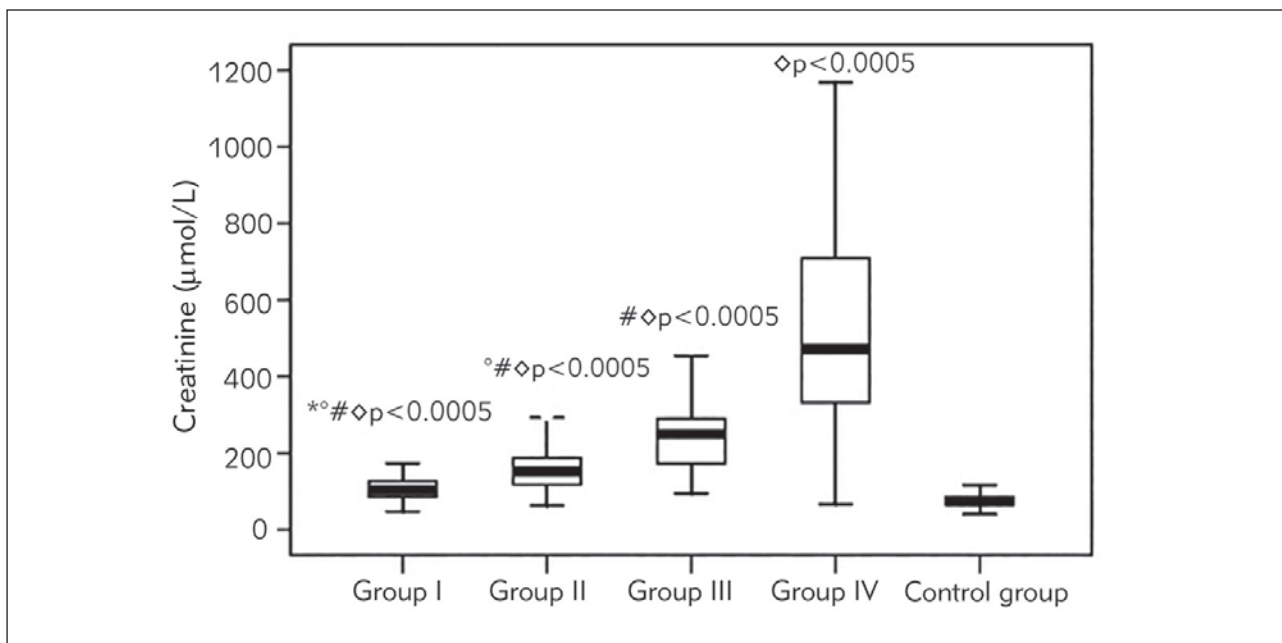
Figure 2 Box-and-whisker plots of creatinine concentration (mmol/L) in the studied groups.
The solid horizontal lines denote the median value, the box represents 25% and 75% interquartile ranges and the whiskers represent minimum and maximum values.
Group I – patients with stage 1 CKD (GFR=60.00–89.99 mL/min/1.73 m²); Group II – patients with stage 2 CKD (GFR=30.00–59.99 mL/min/1.73 m²); Group III – patients with stage 3 CKD (GFR=15.00–29.99 mL/min/1.73 m²); Group IV – patients with stage 4 CKD (GFR 14.99 mL/ min/1.73 m²)
p – probability; NS – not significant; * – compared to Group II; ° – compared to Group III; # – compared to Group IV; ◊ – compared to Control group
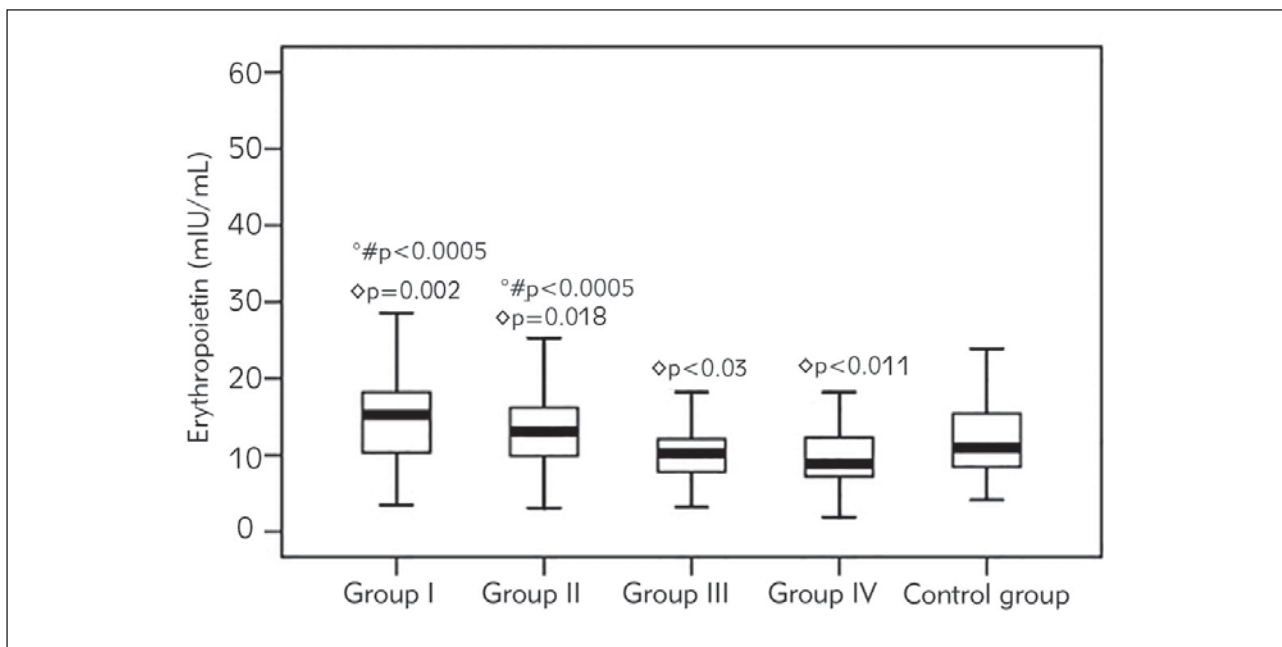
Figure 3 Box-and-whisker plots of erythropoietin concentration (mIU/mL) in the studied groups.
The solid horizontal lines denote the median value, the box represents 25% and 75% of interquartile ranges and the whiskers relate to minimum and maximum values.
Group I – patients with stage 1 CKD (GFR=60.00–89.99 mL/min/1.73 m²); Group II – patients with stage 2 CKD (GFR=30.00–59.99 mL/min/1.73 m²); Group III – patients with stage 3 CKD (GFR=15.00–29.99 mL/min/1.73 m²); Group IV – patients with stage 4 CKD (GFR 14.99 mL/ min/1.73 m²)
p – probability; NS – not significant; * – compared to Group II; ° – compared to Group III; # – compared to Group IV; ◊ – compared to Control group

Table I Correlations between serum glomerular filtration rate (GFR) and clinical laboratory parameters in CKD group and control group.

| Variables | GFR (mL/min/1.73/m^2) | | | |
| | CKD group (n=356) | | Control group (n=206) | |
	rho	p	rho	P
Haemoglobin (g/L)	0.501	0.0001	0.155	0.026
Creatinine (μmol/L)	-0.789	0.0001	-0.532	0.0001
Erythropoietin (mIU/mL)	0.342	0.0001	-0.045	0.517

n – number of cases; rho – Spearman's correlation coefficient; p – probability

in the control group [74.0 μmol/L (65.0–86.3 μmol/L)] (p<0.0005). Median serum creatinine concentration in Group I [104.0 μmol/L (85.3–126.8 μmol/L)] was significantly (p<0.0005) lower than in Group II [152.0 μmol/L (117.5–189.0 μmol/L)], Group III [250.0 μmol/L (172.0–293.0 μmol/L)] and Group IV [471.5 μmol/L (328.8–713.3 μmol/L)], respectively. A significant difference in serum creatinine concentration was also found when Group II was compared with Group III (p<0.0005) and Group IV (p<0.0005), and when Group III was compared with Group IV (p<0.0005) (*Figure 2*).

The median erythropoietin concentrations in the control group [11.0 mIU/mL (8.4–15.4 mIU/mL)] were significantly lower than in Group I [15.3 mIU/mL (10.3–18.2 mIU/mL); (p=0.002)] and Group II [13.1 mIU/mL (9.9–16.2 mIU/mL); (p=0.018)] and significantly higher than median erythropoietin concentrations in Group III [10.2 mIU/mL (7.8–12.2 mIU/mL); (p=0.03)] and Group IV [8.9 mIU/mL (7.1–12.5 mIU/mL); (p=0.011)]. Median erythropoietin concentration in Group I was significantly higher than in Group III (p<0.0005) and Group IV (p<0.0005). A significant difference in erythropoietin concentration was also observed between Group II and III (p<0.0005), and between Group II and IV (p<0.0005). Comparison of the erythropoietin concentrations between other groups showed no significant differences (*Figure 3*).

There was a statistically significant positive correlation between GFR and haemoglobin concentration in the CKD group (rho=0.501; p=0.0001) and control group (rho=0.155; p=0.026). Statistically significant positive correlation was found between GFR and concentration of erythropoietin in CKD group (rho=0.342; p=0.0001), while negative, although not significant correlation was observed between GFR and concentration of erythropoietin in the control group of healthy subjects (rho=-0.045; p=0.517). Statistically significant negative correlation was observed between GFR and serum creatinine concentration in both CKD group (rho=-0.789; p=0.0001) and the control group (rho=-0.532; p=0.0001) (*Table I*).

Discussion

Chronic kidney disease is an important epidemic and public health problem associated with a high risk of vascular disease and cardiovascular mortality, and with kidney disease progression. The prevalence of CKD is so high that it represents a worldwide epidemic and a public health problem all over the world (18). Anaemia is a major co-morbidity of CKD and as kidney disease progresses, anaemia increases in prevalence, affecting nearly all patients with stage 5 CKD. It is associated with reduced quality of life and increased incidence of cardiovascular disease, hospitalizations, cognitive impairment, and mortality (19).

EPO deficiency is the most significant cause of anaemia in CKD and has been demonstrated to occur at each stage of kidney failure. Given that the kidney is the sole source of EPO synthesis in adults, reduction in kidney mass as occurs in progressive CKD often results in impairment of EPO production, resulting in anaemia (20). EPO is a glycoprotein hormone with a molecular mass of 30.4 kDa composed of a single 165 amino acid residues chain to which four glycans are attached (3). EPO is primarily produced by peritubular interstitial cells in the deep cortex and outer medulla of kidneys of the adult and in hepatocytes in the fetus (21). These cells are sensitive to hypoxia and once they are sensed, it leads to an increased EPO production (22). Erythropoietin is essential for terminal maturation of erythrocytes (23). In the confines of the bone marrow, erythroid progenitor cells proliferate into large, nucleated proerythroblasts, which are committed into producing cells of the erythroid series (2). Proerythroblasts are exquisitely sensitive to EPO. They proliferate and develop into erythroblasts and afterwards into reticulocytes that enter the peripheral circulation where they mature into red blood cells (22).

Normal development of erythrocytes and biosynthesis of haemoglobin depend on an optimal biochemical state and adequate supplies of the necessary building blocks including protein, vitamins, hormones, iron and copper. If these components are lacking for a prolonged time, erythropoiesis and biosynthesis of haemoglobin slows and anaemia may

result (2, 24). Anaemia is a common finding in patients with CKD and is primarily due to reduced production of erythropoietin in the kidney and shortened red cell survival (21).

Our study showed that anaemia was already observed below a GFR of 90 mL/min/1.73 m^2. Radtke et al. (25) in their study showed that renal anaemia is manifested in patients with creatinine clearance below 40 mL/min. Fehr et al. showed that the prevalence of anaemia with haemoglobin <110 g/L was around 30 to 40% in patients with severe renal failure and around 15% in patients with clearance of 20 to 39 mL/min (9). The National Health and Nutrition Examination Survey (NHANES) database III was used in two different studies examining the correlation between prevalent haemoglobin concentration and eGFR. Using a cut-off value of 130 g/L in men and 110 g/L in women, the prevalence of renal anaemia increased from 1% at eGFR of 60 mL/min/1.73 m^2 to 90% at eGFR of 30 mL/min/1.73 m^2 and to 33–67% at eGFR of 15 mL/min/1.73 m^2 (12, 26–27). However, NHANES IV using the WHO definition of anaemia showed a lower prevalence of anaemia for each CKD stage (28).

Our study showed that the concentration of haemoglobin decreased with increasing renal failure. The CKD patients in all four groups had significantly lower (p<0.0005) concentrations of haemoglobin compared to the control group. Being below 30 mL/min/1.73 m^2 of GFR (CKD III and IV), the EPO levels were lower than in the control group. This can be attributed to the serious damage of the kidney tissues and the lack of normal EPO biosynthesis, secretion and regulation. Several studies examined the relationship between erythropoietin and haemoglobin levels in patients with various CKD stages. Radtke et al. (25) measured EPO levels in 135 patients. The first group consisted of 117 patients with chronic renal disease, and the second group of 18 patients suffering from end stage renal failure. They established significantly increased average levels of EPO in all groups as compared to the average level of EPO for 59 reference subjects. They also established that levels of EPO decreased with increased deterioration of excretory renal function (25).

Fehr et al. (9) attempted to establish a quantitative association between erythropoietin levels and haemoglobin at different levels of creatinine clearance. A total number of 5120 consecutive patients were investigated. Analysis of erythropoietin and haemoglobin levels showed that patients with creatinine clearance >90 mL/min (group E) and 60–90 mL/min (group A) had identical levels of haemoglobin and erythropoietin. However, EPO levels only rose mildly in patients with creatinine clearance 20–40 mL/min (group B) and 40–60 mL/min (group C) compared to group E, but were equal between groups A and E (9).

Chandra et al. (29) studied 48 children with various degrees of renal insufficiency. They showed a significant correlation of haemoglobin and creatinine clearance below a clearance of 40 mL/min.

In the present study, the serum creatinine levels were significantly higher (p<0.0005) in all stages of CKD patients than in healthy controls. Serum creatinine levels increased with deteriorating renal function, with the highest levels recorded in CKD group IV patients. The median EPO concentrations in group I and II were significantly higher (p=0.002; p=0.018), while median EPO concentrations in group III and IV were significantly lower (p=0.03; p=0.011) than in the control group. In patients with CKD, GFR positively correlated with the concentration of haemoglobin and erythropoietin, while the correlation between GFR and serum creatinine concentration was negative.

Lack of EPO is a primary factor causing anaemia in CKD. The renal fibroblasts lose their EPO-producing capacity after injury and trans-differentiate to scar-producing myofibroblasts (29). The successful correction of CKD anaemia has resulted in reduction of associated morbidity and improvement of functionality, exercise tolerance, cognitive function and overall quality of life. Moreover, significant reduction of cardiovascular morbidity and mortality has occurred. In 2007, the National Kidney Foundation (NKF) and Kidney Disease Outcomes Quality Initiative (KDOQI) Work Group updated the recommended haemoglobin level target between 110 and 120 g/L for all patients with CKD and issued a statement warning about greater risk when targeting haemoglobin levels higher than 130 g/L (31). Also, International Clinical Practice Guideline for Anemia in Chronic Kidney Disease suggests to target haemoglobin levels ≤115 g/L, with individualized consideration of slightly higher haemoglobin targets to improve quality of life (32). Therefore, change in the global approach to CKD from the end-stage renal disease (ESRD) treatment to much more aggressive primary and secondary prevention is an imperative (33).

Conclusion

Erythropoietin biosynthesis and secretion are inversely regulated by haemoglobin levels. A low tissue pO_2 leads to up-regulation of the hypoxia-inducible factor-alpha, a transcription factor that regulates the erythropoietin biosynthesis in the renal cortex. Basically, »relative erythropoietin deficiency« in the context of renal failure can be caused by two different ways: either the biosynthesis of erythropoietin is decreased due to the tissue damage by the underlying disease, or the set-point for erythropoietin secretion is lowered in relation to tissue oxygenation. Our results showed positive correlation between glomerular filtration rate and haemoglobin and erythro-

poietin concentration in patients with chronic kidney disease, while the correlation between glomerular filtration rate and serum creatinine concentration was negative.

Conflict of interest statement

The authors stated that they have no conflicts of interest regarding the publication of this article.

References

1. Wenger RH, Kurtz A. Erythropoietin. In: Comprehensive Physiology, the American Physiological Society, Wiley-Blackwell 2011; 1: 1759–94.

2. McCance KL, Huether SE, Brashers VL, Rote NS. Unit VIII, The Structure and Function of the Hematologic System. In: Pathophysiology, The Biologic Basis for Disease in Adults and Children, 7th ed. Elsevier-Mosby 2014; 954–1082.

3. Jelkmann W. Physiology and Pharmacology of Erythropoietin. Transfus Med Hemother 2013; 40: 302–9.

4. Bunn HF. Erythropoietin. Cold Spring Harb Perspect Med 2013; 3: a011619.

5. Tojo Y, Sekine H, Hirano I, Pan X, Souma T, Tsujita T, et al. Hypoxia Signaling Cascade for Erythropoietin Production in Hepatocytes. Molecular and Cellular Biology 2015; 35(15): 2658–72.

6. Sopić M, Joksić J, Spasojević-Kalimanovska V, Bogavac-Stanojević N, Simić-Ogrizović S, Kravljača M, Jelić Ivanović Z. Downregulation of AdipoR1 is associated with increased circulating adiponectin levels in serbian chronic kidney disease patients. J Med Biochem 2016; 35: 436–42.

7. Erslev AJ, Caro J, Miller O, Silver R. Plasma erythropoietin in health and disease. Ann Clin Lab Sci 1980; 10(3): 250–7.

8. Panjeta M, Tahirovic I, Karamehic J, Sofic E, Ridic O, et al. The relation of erythropoietin towards haemoglobin and hematocrit in varying degrees of renal insufficiency. Mater Sociomed 2015; 27(3): 144–8.

9. Fehr T, Ammann P, Garzoni D, Korte W, Fierz W, et al. Interpretation of erythropoietin levels in patients with various degrees of renal insufficiency and anemia. Kidney Int 2004; 66: 1206–11.

10. MacGinley RJ, Walker RG. International treatment guidelines for anemia in chronic kidney disease – what has changed? Med J Aust 2013; 199(2): 84–5.

11. McCleallan W, Arnof SL, Bolton WK, Hood S, Lorber DL, Tang KL, et al. The prevalence of anemia in patients with chronic kidney disease. Curr Med Res Opin 2004; 20(9): 1501–10.

12. Astor BC, Muntner P, Levin A, Eustace JA, Coresh J. Association of kidney function with anemia: the Third National Health and Nutrition Examination Survey (1988–1994). Arch Intern Med 2002; 162: 1401–8.

13. World Health Organization (WHO). Nutritional anaemias: report of a WHO scientific group. Geneva, Switzerland: World Health Organization, 1968.

14. Simon JF, Nakhoul G. Correction: Anemia of chronic kidney disease. Cleve Clin J Med 2016; 83(10): 739.

15. National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: Evaluation, classification, and stratification. Am J Kidney Dis 2002; 39: S1–266.

16. Vervoort G, Willems HL, Wetzels JFM. Assessment of glomerular filtration rate in healthy subjects and normo-albuminuric diabetic patients: validity of a new (MDRD) prediction equation. Nephrol Dial Transplant 2002; 17(11): 1909–13.

17. Larsen K. Creatinine assay by a reaction-kinetic approach. Clin Chem Acta 1972; 41: 209–17.

18. Mula-Abed W-AS, Al Rasadi K, Al-Riyami D. Estimated Glomerular Filtration Rate (eGFR): A Serum Creatinine-Based Test for the Detection of Chronic Kidney Disease and its Impact on Clinical Practice. Oman Medical Journal 2011; 27(2): 108–13.

19. Journal of the American Society of Nephrology: JASN. Mechanisms of Anemia in CKD. I Am Soc Nephrol 2012; 23(10): 1631–4.

20. Hayat A, Haria D, Salifu MO. Erythropoietin stimulating agents in the management of anemia of chronic kidney disease. Patient Preference and Adherence 2008; 2: 195–200.

21. Nakhoul G, Simon JF. Anemia of chronic kidney disease: Treat it, but not too aggressively. Cleve Clin J Med 2016; 83(8): 613–24.

22. Tsagalis G. Renal anemia: a nephrologist's view. Hippokratia 2011; 15(1): 39–43.

23. Rambod M, Kovesdy CP, Kalantar-Zadeh K. Combined high serum ferritin and low iron saturation in hemodialysis patients: the role of inflammation. Clin J Am Soc Nephrol 2008; 6: 1691–701.

24. Babitt JL, Lin HY. Mechanism of Anemia in CKD. Journal of the American Society of Nephrology 2012; 23(10): 1631–4.

25. Radtke HW, Claussner A, Erbes PM, Scheuermann EH, Schoeppe W, et al. Serum erythropoietin concentration in chronic renal failure: relationship to degree of anemia and excretory renal function. Blood 1979; 54(4): 877–84.

26. Hsu CY, McCulloch CE, Curhan GC. Epidemiology of anemia associated with chronic renal insufficiency among adults in the United States: results from the Third National Health and Nutrition Examination Survey. J Am Soc Nephrol 2002; 13: 504–10.

27. El-Achkar TM, Ohmit SE, McCullough PA, et al. Higher prevalence of anemia with diabetes mellitus in moderate kidney insufficiency: The Kidney Early Evaluation Program. Kidney Int 2005; 67: 1483–8.

28. US Renal Data System: USRDS 2004 Annual Data Report. Bethesda, MD, The National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, 2004.

29. Chandra M, Clemons GK, McVicar MI. Relation of serum erythropoietin levels to renal excretory function: evidence for lowered set point for erythropoietin production in chronic renal failure. J Pediatr 1988; 113(6): 1015–21.

30. Koury MJ. Conversion of Renal Erythropoietin-Producing Cells to Myofibroblasts: A Single Mechanism for Both Anemia and Fibrosis in Kidney Disease. Hematologist 2014; 11; 12–13.

31. Provatopoulou ST, Ziroyiannis PN. Clinical use of erythropoietin in chronic kidney disease: outcomes and future prospects. Hippokratia 2011; 15(2): 109–15.

32. Wyatt CM, Drueke TB. Higher haemoglobin levels and quality of life in patients with advanced chronic kidney disease: no longer a moving target? Kidney Int 2016; 89(5): 971–3.

33. El Nahas MA, Bello AK. Chronic kidney disease: the global challenge. Lancet 2005; 365(9456): 331–40.