RESEARCH ON PLASMA ERYTHROPOIETIN CONCENTRATION IN TYPE 2 DIABETIC PATIENTS WITH KIDNEY INJURY

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Summary

Objectives: To investigate plasma erythropoietin (EPO) concentration and its relationship with some characteristics in type 2 diabetic patients (T2DM) with chronic kidney injury (CKI). ***Subjects and methods:*** A cross-sectional descriptive study on 219 subjects, including 129 patients with type 2 diabetes with chronic kidney injury, 51 patients with diabetes without CKI as a disease control group, and 39 healthy people as a normal control group. All subjects had their plasma EPO levels quantified by ELISA. ***Results:*** The median EPO concentration of the research group was 35.83 (21.55 - 56.34) UI/L, lower than the disease control group, which was 44.72 (37.25 - 63.90) UI/L, and lower than the normal control group, which was 113.31 (30.55 - 378.42) UI/L, $p < 0.001$. The rate of EPO reduction in the study group was 41.9%. EPO concentration was positively correlated with GFR, $r = 0.242$, $p < 0.01$. Creatinine is an independent factor associated with reduced EPO, $p < 0.05$. ***Conclusion:*** The rate of EPO reduction in the type 2 DM group with CKI was 41.9%. Decreases in EPO levels are common in KI patients with type 2 diabetes and are associated with decreased GFR.

* *Keywords:* Type 2 diabetes Mellitus; Renal Injury; GFR; Plasma EPO levels.

INTRODUCTION

Anemia is one of the manifestations in patients with type 2 diabetes. The prevalence and degree of anemia increase with renal complications, especially severe in patients with end-stage diabetic nephropathy. Andrews M. et al. [1] confirmed that anemia is a common manifestation in patients with type 2 diabetes without renal complications. The pathogenesis involves many factors. Autonomic dysfunction and renal hypoxia, even in the absence of renal injury,

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lead to endogenous EPO deficiency [2, 3], especially when ischemic kidney injury is associated with renal failure. Some studies also focused on changes in EPO levels in patients with type 2 diabetes: Panjeta M. et al. [4] concluded that EPO levels decreased in diabetic nephropathy patients compared with controls. Usually, the degree of reduction is positively correlated with the glomerular filtration rate; Fujita Y. et al. [5] confirmed that low EPO levels are seen in diabetic patients and are especially lower in diabetic patients with chronic kidney disease. However, in Vietnam, there have not been many studies on EPO levels in diabetic patients with and without kidney damage. For the above reasons, we carry out the study with 2 objectives:

1. Investigate plasma EPO levels in patients with type 2 diabetes with kidney damage.

2. Find out the relationship between EPO concentration and some characteristics of studied patients.

SUBJECTS AND METHODS

1. Subjects

The research subjects include 219 people divided into 3 groups:

- The research group: 129 people with type 2 diabetes with kidney damage diagnosed and treated at Viet Tiep Hospital, Hai Phong;

- The disease control group: 51 people with type 2 diabetes without kidney damage;

- The normal control group: 39 healthy people of similar age and gender.

** Criteria for selecting study groups:*

- Patients with type 2 diabetes are monitored and treated. All patients had complete follow-up records;

- Age ≥ 40 years old;

- All patients were determined to have KI, including one of the following kidney damage manifestations: Microalbumin in urine: MAU (+), Macroalbumin in urine: MAC (+), and Chronic renal failure;

- Agree to participate in the study.

** Criteria for choosing the disease control group:*

- Patients with type 2 diabetes are monitored and treated. All patients had complete follow-up records;

- Age ≥ 40 years old;

- All patients were determined to have no KI after screening;

- Agree to participate in the study.

** Criteria for choosing the healthy control group:*

- People who go for regular health check-ups are concluded to be healthy;

- Agree to participate in the study.

** Exclusion criteria for study group and disease control group:*

- Patients suffering from an acute illness such as pneumonia, fever, etc.;

- Used recombinant EPO for 2 weeks near the data collection date;

- Women who are menstruating or breastfeeding.

2. Methods

- Study design: Cross-sectional description, comparative statistic.

- Exploiting medical history, used drugs.

- Physical examination to detect disease symptoms.

- Having a full blood count and blood chemistry test.

- Collecting 24-hour urine to determine proteinuria, MAU test

- Calculating the glomerular filtration rate (GFR) according to the EPI-CKD formula.

- Quantification of plasma EPO levels: Collect venous blood from fasting subjects. Anticoagulation is followed by plasma separation. EPO quantification by ELISA method. Unit: UI/L. Place of quantification: Department of Pathophysiology, Vietnam Military Medical University.

- Evaluation of EPO concentrations in the disease group was based on the healthy control group: Patients with decreased EPO levels are defined as < the low value of the quartile of the normal control group.

- Some diagnostic criteria used in the study:

+ MAU (+): As urinary microalbumin concentration > 300 mg/L;

+ MAC (+): As urine analysis samples 10 indicators with proteinuria (+);

+ CKD staging is based on glomerular filtration rate (KDIGO 2012 classification);

+ Diagnosis of anemia based on hemoglobin: Men < 130 g/L, women < 120 g/L.

- Data were processed using SPSS 22.0 software. Graphs are drawn automatically on the computer.

RESULTS AND DISCUSSION

The average age of the research group was 66.68 ± 9.48 years old, the proportion of men accounted for 44.9%; the median time of diagnosed disease is 10 years (6 - 16); 20.4% have MAU (+), 11.6% MAC (+), 68.0% Chronic renal failure; The median GFR was 41.6 mL/min (22.3 - 66.0).

1. EPO concentration characteristics of the study group

Table 1: Comparison of median plasma EPO concentrations in subjects.

Features	Study group (1) (n = 129)	Disease control group (2) (n = 51)	Healthy control group (3) (n = 39)	p
EPO (UI/L) Median (Quarterial)	35.83 (21.55 - 56.34)	44.72 (37.25 - 63.90)	113.31 (30.55 - 378.42)	p < 0.001 p (1)(2) < 0.005 p (1)(3) < 0.001 p (2)(3) < 0.05
Min	6.22	15.47	15.95	
Max	261.33	387.27	612.75	

The median EPO concentration in the study group was the lowest, followed by the disease control group, and the highest was in the healthy control group, the difference was significant, $p < 0.001$. When compared with other studies, we found similarities, both in patients with type 2 diabetes with and without KI. Previous studies have shown that EPO levels are lower in patients with diabetes than in patients without diabetes. Since 2006, Symeonidis A. et al. [6] have quantified EPO levels in the group of diabetic patients compared with the group of non-diabetic patients, and the results show that the median EPO concentration in the diabetic group without anemia is 12 UI/L, significantly lower than the group without diabetes without anemia, $p < 0.001$. In particular, the concentration of EPO in the diabetic group whit anemia was also lower than in the diabetic group without anemia, $p < 0.001$. This suggests that an EPO decrease is already present in patients with diabetes and is also associated with anemia. Several mechanisms have been proposed for low EPO levels in patients with diabetes, including abnormal ischemia sensing due to diabetic autonomic neuropathy, impaired EPO production due to tubulointerstitial lesions, and disorders dysfunction of hypoxia-inducible factors. In the study of Fujita Y. et al. in 2019 [5], it was found that about 60% of diabetic patients without CKD have relative EPO deficiency. EPO deficiency should be considered a factor in anemia of unknown etiology in patients with diabetes, even in those without KI.

Table 2: Comparison of the rate of increase/decrease in EPO levels between the study and disease control group.

Features	Study group (n = 129)		Disease control group (n = 51)		p
	Number of patients	Ratio %	Number of patients	Ratio %	
Decrease	54	41 .9	09	17.6	< 0.005
Normal	75	58.1	41	80.4	
Increase	0	0	10	2.0	

The study group had up to 41.9% (54/129) of patients with reduced EPO concentration compared to healthy people. The proportion of patients with EPO reduction in the study group was significantly higher than in the disease control group (41.9 % versus 17.6%), p < 0.005. Going into the explanation of the decreasing EPO concentration in patients with diabetes with KI, many hypotheses have been proposed. In patients with CKD, a low EPO level corresponds to the degree of anemia. EPO deficiency begins early in the course of CKD, but it appears that when GFR falls below 30 mL/min/1.73 m^2, this deficiency becomes more severe [5]. This absolute EPO deficiency may be due to reduced EPO output and/or an error in EPO sensing.

2. Relationship between plasma EPO concentration and some patient characteristics

Table 3: Association of anemia, EPO levels with the stage of chronic kidney disease (n = 129).

Features		CKD stage 1 + 2 (1), (n = 40) GFR > 60 mL/min		CKD stage 3 + 4 (2), (n = 71) GFR 15 - 60 mL/min		CKD stage 5 (3), (n = 18) GFR < 15 mL/min		p
		n	%	n	%	n	%	
Anemia		16	40.0	52	73.2	16	88.9	< 0.001
EPO (UI/L)	Reduction	14	35.0	25	35.2	15	83.3	< 0.005
	Normal	26	65.0	46	64.8	3	16.7	

The rate of anemia and the rate of reduction of EPO increased with the stage of chronic kidney disease. Deficiency in EPO levels and reduced EPO function are the main causes of anemia. During development, EPO is mainly produced in the fetal liver by hepatocytes [7]. As the site of erythropoiesis changes from the fetal liver during development to the bone marrow at birth, the production of EPO shifts from the fetal liver to the kidney. EPO excitatory receptors (EpoRs) are located on red cell-producing cells. EpoR is expressed at the highest levels on red cell progenitor cells and provides an important regulatory capacity of EPO on red blood cell production. Expression of the extra-hematopoietic erythrocyte adult form of EpoR raises the possibility that EPO activity involved in the survival, proliferation, and differentiation of red blood cells may not be limited during erythrocytosis [7]. Thus, when EPO decreases, it will reduce the transition from immature red blood cells to mature in the bone marrow, causing Hb decrease.

Table 4: Comparison of the median value of RBC, HGB, HCT, and EPO with different kidney disease stage groups (n = 129).

Indexes	**CKD stage 1 + 2 (1), (n = 40) GFR > 60 mL/min**	**CKD stage 3 + 4 (2), (n = 71) GFR 15 - 60 mL/min**	**CKD stage 5 (3), (n = 18) GFR < 15 mL/min**	**p**
RBC (T/l), ($\overline{X} \pm$ SD)	4.20 ± 0.56	3.82 ± 0.67	3.43 ± 0.68	< 0.001
HGB (g/L), ($\overline{X} \pm$ SD)	128.0 ± 20.77	113.09 ± 20.70	99.33 ± 19.34	< 0.001
HCT (L/L), ($\overline{X} \pm$ SD)	0.37 ± 0.05	0.33 ± 0.06	0.30 ± 0.05	< 0.001
EPO (UI/L), Median (Quartile)	37.35 (25.87 - 65.0)	42.09 (22.98 - 57.88)	22.57 (15.39 - 27.03)	p < 0.001 p (1)(2) > 0,05 p (1)(3) < 0.001 p (2)(3) < 0.001

The mean value of RBC, HGB, and HCT decreased gradually according to the severity of the CKD stage, with statistical significance, $p < 0.001$. The median value of EPO decreased gradually according to the severity of the CKD stage, with statistical significance, $p < 0.001$. However, there was no difference in the group of patients with CKD stages 1 + 2 and 3 + 4, $p > 0.05$. The results of our study are consistent with the literature on the role of reducing EPO secretion in the presence of renal parenchymal damage and are consistent with the research results of Fujita Y. et al. in 2019 [5]. The potential cause of low EPO levels in patients with diabetic nephropathy, which declines as GFR decreases, is explained by several mechanisms. The reasons why EPO levels do not increase in response to anemia have not been elucidated. One possibility is a decrease in the amount of specific synthetic EPO in the renal tubule cells upon injury. Another type is disruption of renal interstitial anatomy or vascular structures that interfere with oxygen sensing through hypoxia-inducible transcription factor 1-alpha (HIF-1-alpha) [5].

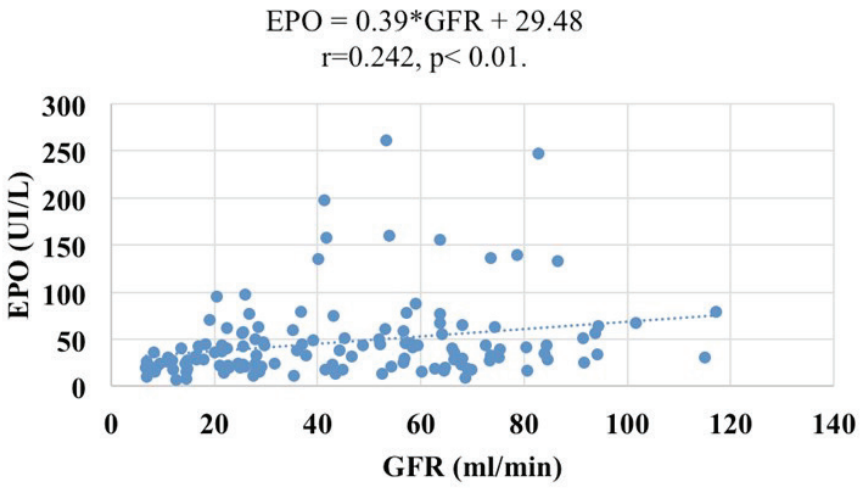

Figure 1: Correlation of plasma EPO concentration with GFR (n = 129).

There was a significant positive correlation between plasma EPO concentration and GFR in the study group, $p < 0.01$. Regarding the correlation between GFR and EPO, Panjeta M. et al. in 2017 [4] also gave similar results: (The control group of 206 healthy people: There was no significant correlation between EPO concentrations with GFR; while in the group of 356 patients with

CKD, the correlation coefficient r = 0.342, p = 0.000). Patients with impaired renal function showed significantly lower EPO concentrations at baseline. It is conceivable that pro-inflammatory cytokines and advanced glycation end products act at least in part through NF-kappa-B, interfering with the specific synthesis of EPO by renal interstitial cells [4].

Table 5: Multivariate analysis of independent factors associated with reduced plasma EPO.

Factors	Odds ratio (OR)	CI 95%	p
HbA1C	0.902	0.779 - 1.044	> 0.05
Ure	0.938	0.853 - 1.032	> 0.05
Creatinine	1.008	1.002 - 1.014	< 0.05

Only serum creatinine was an independent factor associated with a reduction in plasma EPO levels in type 2 DM patients with KI, p < 0.05. Thus, in this study, we found that GFR has a role in assessing the association as well as predicting EPO reduction. According to the pathogenesis and the fact of published research results, the decrease in EPO concentration in patients with CKD and the degree of reduction in EPO is related to the decrease in GFR in this group of patients [4, 8]. In patients with type 2 diabetes with chronic kidney disease, this result is also confirmed [5]. In patients with type 2 diabetes with anemia, the study of Fujita Y. et al. demonstrated the role of EPO in maintaining renal function. The above authors found that low EPO levels were observed in 73% of anemic patients and 59% of anemic patients even without CKD, suggesting that EPO deficiency precedes the onset of CKD in DM patients. Multivariate analysis showed that iron status and hemoglobin levels were the major determinants of EPO levels. The results of this study also suggest that low EPO levels, not low hemoglobin levels, are associated with a faster reduction in GFR. The decline in GFR is more rapid, especially when EPO levels are below the upper limit of normal. Lower EPO levels are associated with a rapid decrease in GFR, particularly in iron-deficient patients. Low EPO levels, especially when accompanied by poor

iron status, are predictive of rapid loss of kidney function. This conclusion shows that there is a 2-way relationship between EPO and renal function, with decreased renal function causing a decrease in EPO and a decrease in EPO leading to a decrease in renal function.

CONCLUSION

- The median EPO concentration of the type 2 DM group with KI was 35.83 (21,55 - 56,34) UI/L, lower than the type 2 DM group without KI group 44.72 (37.25 - 63.90) UI/L, and lower than the healthy control group 113.31 (30.55 - 378.42) UI/L, $p < 0.001$. The rate of EPO reduction in the type 2 DM group with CKI was 41.9%.

- EPO concentration was positively correlated with GFR, $r = 0.242$, $p < 0.01$. Creatinine is an independent factor associated with reduced EPO in type 2 DM patients with KI, $p < 0.05$.

REFERENCES

1. Andrews M., Arredondo M. (2012). Ferritin levels and hepcidin mRNA expression in peripheral mononuclear cells from anemic type 2 diabetic patients. *Biological Trace Element Research;* 149(1):1-4.

2. Barbieri J., Fontela P.C., Winkelmann E.R., et al. (2015). Anemia in Patients with Type 2 Diabet es Mellitus. *Anemia;* 2015:354737.

3. Deray G., Heurtier A., Grimaldi A., et al. (2004). Anemia and diabetes. *Am J Nephrol;* 24(5):522-526.

4. Panjeta M., Tahirović I., Sofić E., et al. (2017). Interpretation of Erythropoietin and Haemoglobin Levels in Patients with Various Stages of Chronic Kidney Disease. *J Med Biochem;* 36(2):145-152.

5. Fujita Y., Doi Y., Hamano T., et al. (2019). Low erythropoietin levels predict faster renal function decline in diabetic patients with anemia: A prospective cohort study. *Sci Rep;* 9(1):14871.

6. Symeonidis A., Kouraklis-Symeonidis A., Psiroyiannis A., et al. (2006). Inappropriately low erythropoietin response for the degree of anemia in patients with noninsulin-dependent diabetes mellitus. *Annals of hematology;* 85:79-85.

7. Jelkmann W. (2011). Regulation of erythropoietin production. *J Physiol*; 589(6):1251-1258.

8. Pappa M., Dounousi E., Duni A., et al. (2015). Less known pathophysiological mechanisms of anemia in patients with diabetic nephropathy. *Int Urol Nephrol;* 47(8):1365-1372.