Medico Research Chronicles

ISSN No. 2394-3971

Original Research Article

SERUM ERYTHROPOIETIN LEVEL CORRELATED WITH MICROALBUMINURIA IN DIABETIC PATIENTS

Mehmet Yamak[1], Süleyman Ahbab[1*], Meltem Gürsu[2], Yesim Kara[1], Esra Ataoğlu[1], Savaş Öztürk[2], Fuat Şar[1]

1. Internal Medicine Clinic, Haseki Training and Research Hospital, Istanbul, Turkey
2. Nephrology Clinic, Haseki Training and Research Hospital, Istanbul, Turkey

Submitted on: April 2016
Accepted on: April 2016
For Correspondence
Email ID:
drsahbab@hotmail.com

Abstract

Erythropoietin has beneficial effects in diabetic nephropathy, neuropathy and retinopathy. We investigated the relationship between iron indices and erythropoietin levels in type 2 diabetics with microvascular complications. Type 2 diabetic patients with microalbuminuria, diabetic retinopathy (DRP) and neuropathy were included to the present study. Serum erythropoietin levels and iron indices were recorded besides demographic, clinic and biochemical data. Of the 59 patients included in this study, microalbuminuria was present in 29 patients (49.2 %), while DRP and diabetic neuropathy were detected in 23 (39.7%) and 20 (34.5%) patients, respectively. Erythropoietin concentration (13.17±4.48 mU/mL vs 15.77±5.16 mU/mL; p=0.04) and total iron binding capacity (TIBC) of the microalbuminuric group were lower than those without microalbuminuria, while iron levels were similar. Erythropoietin concentration was lower in patients with DRP compared to those without DRP (12.84±4.95 mU/mL vs 15.49±4.82 mU/mL; p=0.04). TIBC was higher in patients with diabetic neuropathy; while erythropoietin (13.93±4.83 mU/mL vs. 14.71±5.13 mU/mL; p=0.57), ferritin and iron levels were similar to patients without neuropathy. Lower erythropoietin concentrations observed in patients with DRP and neuropathy are consistent with the hypothesis that erythropoietin deficiency may precede microvascular complications in diabetic patients. Serum erythropoietin level was found to correlate with microalbuminuria (p: 0.006, r:-0.50) but not retinopathy and neuropathy.

Keywords: Erythropoietin, Diabetes Mellitus, Microalbuminuria, Diabetic Retinopathy, Diabetic Neuropathy.

Introduction

Type 2 diabetes mellitus (DM) is one of the leading health problems worldwide with serious morbidity and mortality rates. Good metabolic control is of prime importance in preventing the long term complications of DM. Inflammation, oxidative stress and insulin resistance have

key roles in the emergence of DM. Body iron stores have been shown to be related to insulin resistance. Free iron is a strong pro-oxidant, with active roles in oxidative stress, the formation of free radicals, lipid peroxidation and endothelial dysfunction [1]. Iron causes insulin resistance and hyperinsulinemia by acting on the liver and peripheral tissues. Healthy individuals with higher serum ferritin levels were shown to be at higher risk of developing type 2 DM [2]. Moreover, iron chelation therapy and phlebotomy have been found to attenuate insulin resistance and improve metabolic control in type 2 diabetic patients with elevated serum ferritin concentrations [3]. In addition, studies have shown a relationship between diabetic nephropathy (DNP) and iron indices. Iron and ferritin concentrations were reported to be higher in patients with than without DNP [4].

Erythropoietin (EPO), the main regulator of erythropoiesis, has been shown to stimulate the proliferation and differentiation of erythroid progenitor cells, and to inhibit apoptosis. EPO was also reported to have neuroprotective activities (5). Iron indices and erythropoietin levels have been associated with diabetic neuropathy and retinopathy [6,7]. Treatment with erythropoietin has shown beneficial effects in models of diabetic nephropathy, neuropathy and retinopathy [8,9]. To expand upon these findings, we investigated the relationship between iron indices and erythropoietin levels, and microvascular complications in patients with type 2 DM.

Patients and Methods

Patients diagnosed with type 2 DM according to the criteria of the American Diabetes Association were included in the study. Patients with hepatitis; major neurological, urological, endocrine diseases other than DM; malignancy; renal failure; acute/chronic infectious or inflammatory diseases; chronic obstructive pulmonary disease; and disorders in iron metabolism (i.e., iron deficiency, hemochromatosis, or anemia) were excluded. In addition, patients with a history of iron treatment or blood transfusion, major cardiovascular disease within the last six months, or a history of smoking or alcohol use were excluded. Patient demographic parameters (age, gender), co-morbidities, history of smoking and alcohol use, medications being taken and microvascular complications (e.g. microalbuminuria, retinopathy, neuropathy) were recorded. Body mass index was calculated as weight (kg) / (height)^2 (m^2). Written informed consent for participation in the study was obtained from participants.

Patients with albuminuria>30 mg/day were regarded as having DNP, unless there were other factors that could affect renal function or could interfere with the detection of albumin in urine (e.g. urinary infections). Diabetic retinopathy (DRP) was based on the reports of eye examinations performed within the previous six months. Diabetic neuropathy was based on electromyography within the past year; symptoms alone were not regarded as diagnostic. Serum and plasma samples were obtained from all participants after 12 hours of fasting, and were kept at -80ºC. Fasting blood glucose (FBG), urea, creatinine, iron, ferritin, HbA1c, and hemoglobin concentrations, as well as total iron binding capacity (TIBC), hematocrit, mean corpuscular volume (MCV) and leukocyte and platelet counts were measured. Biochemical analysis was performed using an Architect c16000 analyzer with appropriate methods. HbA1c concentrations were measured by a high pressure liquid chromatography method using a TOSOH C7 analyzer, and hematologic parameters with a Horiba ABX pentra DX 120 machine. Microalbumin, protein and creatinine concentrations in 24 hour urine samples were measured by immunoturbidometric

methods (ABBOTT C1600). Patients were told to abstain from heavy exercise during the day the urine was collected. Glomerular filtration rate was calculated using the CKD-EPI formula. Serum erythropoietin concentrations were determined by immunoassays (Immulite DPC kits; Bio DPC, USA).

Statistical analyses were performed using the SPSS (Statistical Package for Social Sciences) for Windows 16.0 package program. Numerical variables were presented as mean±standard deviation. Quantitative parameters of normal and non-normal distributions were compared using Student's t tests and Mann Whitney U tests, respectively. Non-numerical parameters of normal and non-normal distributions were compared using the Chi-square and Fisher's exact tests, respectively. The Pearson test was used for correlation analysis. Results were evaluated with 95% confidence intervals; p values below 0.05 were regarded as statistically significant.

Results

Of the 59 patients included in this study, 28 (47.5%) were male and 31 (52.5%) female. Their mean age and mean duration of DM were 59.1±10.3 years and 13.10±5.04 years, respectively. Thirty-nine patients (66.1%) were taking insulin and oral antidiabetic drugs, while 20 (34%) were taking only oral antidiabetic drugs. The major co-morbidities present at the time of evaluation were hypertension (n=19, 32.1%), dyslipidemia (n=6, 6.8%), and both (n=12, 27.1%), whereas 20 patients (34%) had no co-existent disease. Microalbuminuria was present in 29 patients (49.2%), while DRP and diabetic neuropathy were detected in 23 (39.7%) and 20 (34.5%) patients, respectively. The mean BMI was 27.8±2.9 kg/m^2. Erythropoietin concentration was negatively correlated with hemoglobin concentration (r=-0.338; p=0.009), hematocrit (r=-0.338; p=0.009) and microalbuminuria (r=-0.427; p=0.001). Median microalbuminuria was 27 mg/day (range, 0.6 mg/day to 300.4 mg/day). Patients were divided into two groups, consisting of 29 patients (12 males, 17 females) with microalbuminuria and 30 (16 males, 14 females) without microalbuminuria, (Table 1).

Table 1. Comparison of patients with and without microalbuminuria

	MA (+) (n=29)	**MA (-) (n=30)**	**P**
	Mean ± Standart Deviation		
Age (years)	58.79±9.10	58.13±9.52	0.78
DM duration (years)	14.07±4.34	12.17±5.53	0.15
Fasting blood glucose (mg/dL)	156.16±40.10	141.27±37.89	0.15
HbA1c (%)	7.70±1.03	7.36±1.23	0.25
Hemoglobin (mg/dL)	13.58±0.76	14.03±1.33	0.12
Hematocrit (%)	40.65±2.20	41.45±3.46	0.30
Mean corpuscular volume (fL)	87.21±3.88	87.27±3.95	0.94
Glomerular filtration rate (mL/min)	85.3±17.7	90.5±16.1	0.25
Creatinine (mg/dL)	0.84±0.17	0.80±0.19	0.43
Body mass index (kg/m^2)	27.85±2.81	27.71±3.03	0.85
Iron (µg/dL)	67.01±23.65	67.96±20.44	0.86

Total iron binding capacity (mcg/dL)	288±36.74	309±39.10	**0.03**
Ferritin (ng/mL)	50.95±29.70	39.61±21.47	0.09
Erythropoietin (mU/mL)	13.17±4.48	15.77±5.16	**0.04**

MA: microalbuminuria

The erythropoietin concentration and TIBC of the microalbuminuric group were significantly lower than those of the group without microalbuminuria, while their serum iron levels were similar. Ferritin concentration was higher in the microalbuminuric group, although the difference was not statistically significant. In the microalbuminuric group, erythropoietin concentration was negatively correlated with hemoglobin concentration (r=-0.401; p=0.03) and microalbuminuria (r=-0.500; p=0.006) (Figure 1).

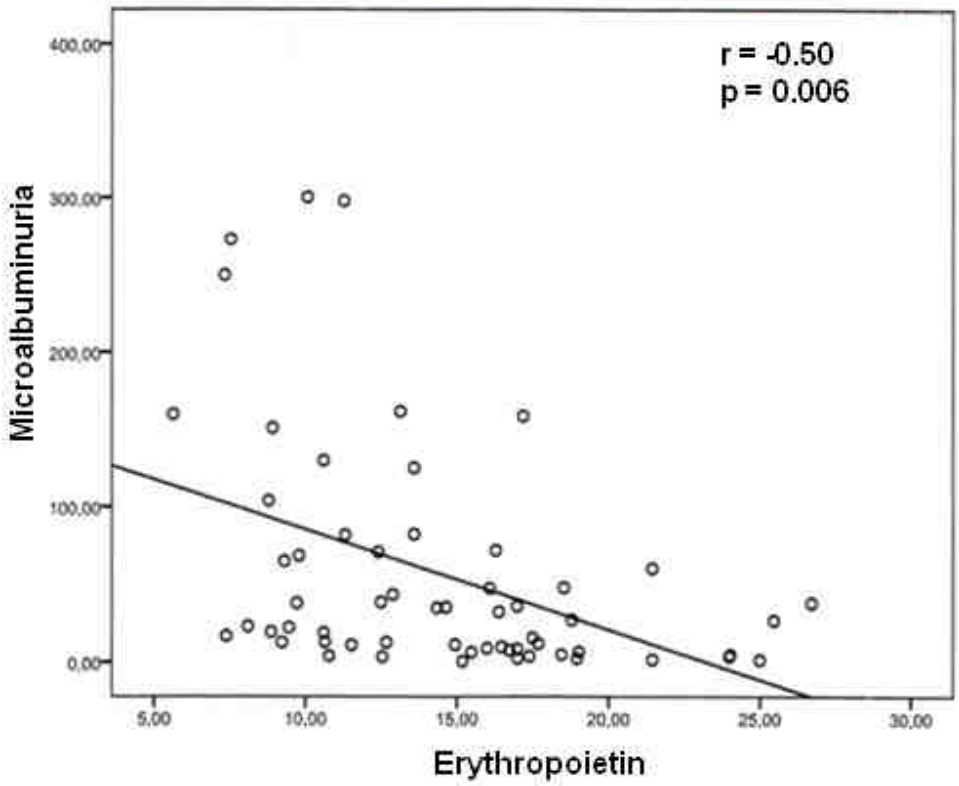

Figure 1. Correlation between microalbuminuria and erythropoietin in diabetic patients

Twenty patients, 8 males and 12 females, were found to have diabetic neuropathy. After excluding one patient because of indeterminate markers of neuropathy, we found that DM duration, FBG, HbA_{1c}, TIBC and glomerular filtration rate were higher in patients with than without diabetic neuropathy (Table 2). DRP was detected in 23 patients, including 10 males and 13 females. After excluding one patient who could not undergo an eye examination, we found that DM duration and FBG levels were higher, and erythropoietin concentration lower, in patients with than without DRP (Table 3).

Table 2. Comparison of patients with and without diabetic neuropathy

	Neuropathy(+) (n=20)	Neuropathy (-) (n=38)	P
	Mean ± Standart Deviation		
Age (years)	58.45±8.27	58.68±9.84	0.92
DM duration (years)	15.25±4.73	12.08±4.93	**0.02**
Fasting blood glucose (mg/dL)	168±39.57	137.63±35.78	**0.004**
HbA_{1c} (%)	7.91±1.10	7.28±1.09	**0.04**
Hemoglobin (g/dL)	13.71±0.94	13.88±1.19	0.58
Hematocrit (%)	40.97±2.39	41.19±3.18	0.78
Mean corpuscular volume (fL)	87.80±4.40	86.97±3.67	0.45
Iron (µg/dL)	63.80±18.48	70.03±23.41	0.37
Total iron binding capacity (mcg/dL)	282.15±38.10	305.76±36.63	**0.02**
Ferritin (ng/mL)	41.91±28.06	47.42±25.76	0.45
Erythropoietin (mU/mL)	13.93±4.83	14.71±5.13	0.57
Glomerular filtration rate (ml/min)	93.55±13.56	84.07±17.03	**0.03**
Microalbuminuria (mg/day)	69.10±72.18	50.96±77.68	0.39
Creatinine (mg/dL)	0.77±0.18	0.85±0.18	0.12
BMI (kg/m^2)	28±3.12	27.68±2.85	0.70

Table 3. Comparison of patients with and without diabetic retinopathy

	DRP (+) (n=23)	DRP (-) (n=35)	P Value
	Mean ± Standart Deviation		
Age (years)	60.7±9.1	57.2±9.2	0.16
DM duration (years)	15.4±5.0	11.7±4.6	**0.005**
Fasting blood glucose (mg/dL)	163±42	138±35	**0.02**
HbA_{1c} (%)	7.79±1.27	7.30±0.99	0.10
Hemoglobin (g/dL)	13.94±0.84	13.74±1.25	0.50
Hematocrit (%)	41.4±2.7	40.9±3.1	0.60
Mean corpuscular volume (fL)	86.9±4.1	87.5±3.8	0.54
Glomerular Filtration Rate (ml/min)	86.0±17.9	89.2±16.7	0.493
Creatinine (mg/dL)	0.84±0.19	0.80±0.18	0.4
BMI (kg/m^2)	27.7±2.9	27.8±3.0	0.88
Iron (µg/dL)	68.8±26.2	67.3±18.9	0.8
Total iron binding capacity (mcg/dL)	290±38	302±38	0.25
Ferritin (ng/mL)	45.3±24.5	45.6±28.0	0.96
Erythropoietin (mU/mL)	12.84±4.95	15.49±4.82	**0.04**
Microalbuminuria (mg/day)	90.5±100.4	35.3±42.9	**0.006**

DRP: diabetic retinopathy

Discussion

Many mechanisms have been proposed for the development and progression of microvascular complications of DM. Body iron stores have been shown to be related to insulin resistance in type 2 DM and individuals with high ferritin concentrations were reported to be at higher risk of developing type 2 DM. Moreover, DNP was reported to have a negative effect on iron metabolism, and vice versa.

Erythropoietin is a glycoprotein hormone produced by cortical interstitial cells of the kidneys. Erythropoietin concentration has been reported to be negatively correlated with hemoglobin concentrations in patients with anemia. However, the low erythropoietin concentration observed in patients with anemia is likely due to chronic diseases including renal failure [10,11]. Anemia is seen at an earlier stage of chronic kidney disease in diabetic than in non-diabetic subjects, and low erythropoietin concentrations have been observed in diabetic patients with normal renal function [12]. Many studies have assessed the relationship between erythropoietin and microvascular complications of DM [13-15]. We therefore sought to evaluate the relationship of erythropoietin concentration and body iron stores with DNP, DRP and diabetic neuropathy.

We found that TIBC and erythropoietin concentrations were lower in patients with than without microalbuminuria, and that the levels of microalbuminuria and erythropoietin in these subjects were negatively correlated. Low erythropoietin concentrations in diabetic patients with microalbuminuria may be regarded as a harbinger of the development of overt nephropathy [16]. TIBC was found to be higher in patients with than without diabetic neuropathy. Other parameters of iron metabolism, however, did not differ significantly in these two groups. Relatively little is known about the relationship between body iron stores and diabetic neuropathy. Histopathologic examination of 22 patients with diabetic neuropathy showed necrotizing vasculitis at perineural and endoneural blood vessels in 6, endoneural hemorrhage in 5, and ferric iron deposits without erythrocytes in the endoneurium in 7 [17]. However, ferric deposits may be associated with hemorrhages due to vascular involvement.

Erythropoietin may have protective effects on the brain and retina by an autocrine or paracrine mechanism [18]. Disordered erythropoietin metabolism has been reported to precede clinically proven diabetic neuropathy. The significant correlations between diabetic neuropathy and fasting blood glucose and HbA_{1c} concentrations suggest that control of diabetes is necessary for the prevention of diabetic neuropathy.

The relationship between BMI and body iron stores is unclear, with some studies showing a positive correlation between ferritin concentration and BMI and others showing no correlation [19,20]. The prevalence of obesity has been reported to be higher in patients with higher ferritin levels [21,22]. In contrast, we observed no correlation between these two parameters in our patients.

Conclusion

Lower erythropoietin concentrations observed in patients with DRP and neuropathy are consistent with the hypothesis that erythropoietin deficiency may precede microvascular complications in diabetic patients. Serum erythropoietin level was observed to correlate with microalbuminuria (p: 0.006, r:-0.50) but not retinopathy and neuropathy.

References

1. Araujo JA, Zhang M, Yin F. Heme Oxygenase-1, Oxidation, Inflammation,

and Atherosclerosis. Front Pharmacol 2012; 3:119.
2. Canturk Z, Cetinarslan B, Tarkun I, Canturk NZ. Serum ferritin levels in poorly- and well-controlled diabetes mellitus. Endocr Res 2003;29(3):299-306.
3. Yu FJ, Huang MC, Chang WT, et al. Increased Ferritin Concentrations Correlate with Insulin Resistance in Female Type 2 Diabetic Patients. Ann Nutr Metab 2012;61(1):32-40.
4. Tajima S, Ikeda Y, Sawada K, et al. Iron reduction by deferoxamine leads to amelioration of adiposity via the regulation of oxidative stress and inflammation in obese and type 2 diabetes KKAy mice. Am J Physiol Endocrinol Metab 2012;302(1):77-86.
5. Hernández C, Simó R. Erythropoietin produced by the retina: its role in physiology and diabetic retinopathy. Endocrine 2012;41(2):220-226.
6. Liu Q, Sun L, Tan Y, Wang G, Lin X, Cai L. Role of iron deficiency and overload in the pathogenesis of diabetes and diabetic complications. Current Medicinal Chemistry 2009;16:113-129.
7. Doggrell SA. A neuroprotective derivative of erythropoietin that is not erythropoietic. Expert Opin Investig Drugs. 2004; 13(11):1517-1519.
8. Spallone V, Maiello MR, Kurukulasuriya N, et al. Does autonomic neuropathy play a role in erythropoietin regulation in non-proteinuric Type 2 diabetic patients? Diabet Med 2004;21(11):1174-1180.
9. Schmidt RE, Green KG, Feng D, et al. Erythropoietin and its carbamylated derivative prevent the development of experimental diabetic autonomic neuropathy in STZ-induced diabetic NOD-SCID mice. Exp Neurol 2008;209(1):161-170.
10. Symeonidis A, Kouraklis-Symeonidis A, Psiroyiannis A, et al. Inappropriately low erythropoietin response for the degree of anemia in patients with noninsulin-dependent diabetes mellitus. Ann Hematol 2006;85:79-85.
11. Maiese K. Erythropoietin and diabetes mellitus. World J Diabetes 2015 Oct 25;6(14):1259-73. doi: 10.4239/wjd.v6.i14.1259
12. Bosman DR, Winkler AS, Marsden JT, Macdougall IC, Watkins PJ. Anemia with erythropoietin deficiency occurs early in diabetic nephropathy. Diabetes Care 2001;24(3):495-499.
13. Mojiminiyi OA, Abdella NA, Zaki MY, El Gebely SA, Mohamedi HM, Aldhahi WA. Prevalence and associations of low plasma erythropoietin in patients with Type 2 diabetes mellitus. Diabetic Medicine 2006;23:839-844.
14. Cosimo M, Brunoa T, Sciaccaa C, et al. Circulating erythropoietin in microalbuminuric type 2 diabetic patients with normal renal function: a pilot study. J Diabetes Complications 2006;20:376-379.
15. Singh DK, Winocour P, Summerhayes B, Viljoen A, Sivakumar G, Farrington K. Are low erythropoietin and 1,25-dihydroxyvitamin D levels indicative of tubulo-interstitial dysfunction in diabetes without persistent microalbuminuria? Diabetes Res Clin Pract 2009;85(3):258-264.
16. Ronald G Pearl. Erythropoietin and organ protection: lessons from negative clinical trials. Crit Care 2014; 18(5): 526.
17. Said G, Lacroix C, Lozeron P, Ropert A, Planté V, Adams D. Inflammatory vasculopathy in multifocal diabetic neuropathy. Brain 2003;126(Pt 2):376-385.
18. Bartnicki P, Kowalczyk M, Rysz J. The influence of the pleiotropic action of erythropoietin and its derivatives on

nephroprotection. Med Sci Monit 2013; 22 (19): 599-605.
19. Iwasaki T, Nakajima A, Yoneda M, et al. Serum ferritin is associated with visceral fat area and subcutaneous fat area. Diabetes Care 2005;28(10):2486-2491.
20. Gillum RF. Association of serum ferritin and indices of body fat distribution and obesity in Mexican American men: the Third National Health and Nutrition Examination Survey. Int J Obes Relat Metab Disord 2001;25(5):639-645.
21. Fernandez-Real JM, Ricart-Engel W, Arroyo E, et al. Serum ferritin as a component of the insulin resistance syndrome. Diabetes Care 1998;21:62-68.
22. Gradinaru D, Margina D, Ilie M, Borsa C, Ionescu C, Prada GI. Correlation between erythropoietin serum levels and erythrocyte susceptibility to lipid peroxidation in elderly with type 2 diabetes. Acta Physiol Hung 2015; 102 (4): 400-8.