Original Article

Association of Erythropoietin, Adiponectin and Leptin levels with Anemia in uremic diabetic patients (Under hemodialysis):

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Summary:

Background: End stage renal disease in patients with diabetes mellitus has been called a medical catastrophe of worldwide dimensions .It was recognized that anemia is a complication of diabetes, particularly in patients with diabetic kidney diseases. The purpose of the present study is to determine whether erythropoietin, adiponectin, and leptin levels correlate with anemia in uremic diabetic patients (under hemodialysis).

Patients and Methods: The studied groups were comprised of (30) diabetic patients(9 patients with type1 and 21 type2 diabetes) with renal failure (group2) who had been under hemodialysis compared with (30) healthy controls (group1). Blood film was obtained in addition to blood tests include fasting serum glucose (FSG), glycosylated hemoglobin (A1c), insulin, urea, creatinine, uric acid, estimated glomerular filtration rate (eGFR), serum erythropoietin (EPO), Adiponectin (ADPN),and leptin.

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Results: It was found that uremic diabetic patients in group2(G2) had higher FSG, A1c, insulin and higher levels of urea, creatinine, uric acid and lower levels of eGFR when compared to healthy controls ($P < 0.05$). Anemia was confirmed in the diabetic patients by Hemoglobin (Hb) and hematocrit (Hct) that were lower than healthy control (group1) (G1) ($P<0.05$).EPO, ADPN levels were increased in uremic diabetic patients as compared to control subjects ($P < 0.05$). While, leptin levels were higher in type2 diabetes when compared to type1 diabetes and control subjects (group1) ($P < 0.05$). There were positive significant correlations between (FSG and A1c), (Hb and Hct), (EPO and leptin), (EPO and Hct), (ADPN and urea). In addition there were significant negative correlations between (FSG and EPO), (A1c and leptin), (ADPN and leptin).Creatinine was negatively associated with Hb and Hct. ADPN was negatively associated with Hb in uremic diabetics.

Conclusions: Anemia is a common accompaniment to diabetes, particularly in those with renal failure or under hemodialysis .Adipocytokines like leptin and adiponectin may involved with anemia in these patients. Leptin in the present study was positively associated with EPO levels (the hormone that stimulates red blood cells production in the bone marrow). ADPN was negatively associated with Hb. These elevated levels of ADPN may antagonize EPO and leptin to increase the hemopoiesis rate and Hb levels in uremic diabetic patients in addition to other factors involved with diabetes.

Keywords: Diabetes, renal failure, anemia, hemodialysis, leptin, adiponectin, erythropoietin.

Introduction:

Diabetes is the most prevalent cause of renal failure that may lead to renal anemia. In addition, anemia may develop earlier in diabetes patients than in patients with renal impairments from other causes [1]. Anemia has a negative impact of patients' survival and is considered an important cardiovascular risk factor associated with renal diseases. In diabetic patients, anemia is the result of diminished Erythropoietin (EPO) production (a hormone produced by the kidneys that stimulates red blood cells production in the bone marrow), and to a lesser degree of increased excretion of EPO in urine whereas EPO responsiveness remains unchanged. It was found that a normochromic, normocytic anemia can occur before evidence of renal impairment is present [2, 3]. Studies have identified anemia as a risk for the need of renal replacement therapy in diabetes. In addition a lower hemoglobin (Hb) level is significantly associated with more rapid decline in the Glomerular filtration rate (GFR). Furthermore, treating anemia early in renal failure has been demonstrated to slow the rate of decline of renal function [3]. During hematopoiesis, there are many growth factors, which stimulate the proliferation and maturation of erythroid progenitors, the main one is erythropoietin (EPO), which act in concert with other growth factors [4]. It was suggested that the obesity (*ob*) gene protein known as leptin (from Greek word leptos "thin") could be involved in early stages of erythropoiesis and stimulate hematopoietic stem cells

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in vitro. The proliferative effects of leptin on hematopoietic stem cells suggesting a role for leptin in erythropoiesis [5]. Adiponectin (ADPN) is an adipocyte-derived hormone, involved in glucose, lipid metabolism and has insulin sensitizing properties. It has a potential protective role for the cardiovascular system. Plasma ADPN levels were decreased and were negatively related to the severity of cardiovascular injury in patients with coronary artery diseases and with type2 diabetes [7]. Patients with ESRD and low plasma ADPN levels had a high risk of cardiovascular death [8]. Previous Studies suggest that certain cytokines might have negative effects on normal hematopoietic cells; for example, the elevations of pro-inflammatory cytokine tumor necrosis factor a (TNFa) and ADPN in certain leukemias and depression of normal bone marrow hematopoiesis [9]. Another study indicated that adipocyte derived proteins leptin and ADPN were related to hemopoiesis, therefore it has shown the possible existence of adipose tissue/bone marrow function linkage [10].

Patients, Materials and Methods:

Thirty diabetic patients and 30 age matched healthy controls were enrolled in this study. Healthy subjects (group1 or G1) were selected from the staff of AL-Kindy and Baghdad Colleges of Medicine. The diabetic patients (group2 or G2) were referred from dialysis unit at Baghdad teaching hospital; patients who had active or chronic infection were excluded. In addition, patients who were taking Human recombinant Erythropoietin treatment were excluded from the study. Group2 was segregated according to type of diabetes and divided into type1 and type2 as (G2 T1 and G2 T2). Male to female ratio was (15/15) in group1 and (20/10) in group2. Serum EPO, ADPN, leptin, and insulin concentrations were measured using a commercially available (Enzyme linked Immunosorbant Assay) ELISA kits (DRG International, Inc., USA).Each kit utilize a principle based on polyclonal antibody to recombinant human EPO, ADPN, leptin, and insulin respectively. Glycosylated hemoglobin (A1c); measured by variant hemoglobin program utilize the principles of ion exchange high performance liquid chromatography (HPLC)). Blood film, hemoglobin, and Hematocrit, in addition to serum urea, creatinine, uric acid, and fasting glucose were measured by enzymatic methods using kits supplied by (Biomegreb, Tunisia). Glomerular filtration rate was estimated (eGFR) using the modification of diet in renal disease (MDRD) abbreviated equation [11]. Determination of hemoglobin was based on conversion of Hb into cyannohemoglobin using drabkin reagent kit (Crescent Diagnostics, Saudi Arabia).Hb was recorded for estimating the risk of anemia. Anemia was defined as hemoglobin (Hb ≤11 g/dl, irrespective of sex) according to the guidelines of the World Health Organization (WHO) [1]. Duration of each hemodialysis session was four hours. Body mass index (BMI) was calculated using the standard formula (post dialysis –weight in kilograms /height in square meters (Kg/m^2) [4].

Statistical methods:

Statistical analysis was performed using SPSS for windows version (12.0). Data were expressed as Mean ± Standard Deviation (X±SD). The least significant difference (LSD) method was used to compare individual groups and for comparison among type1, type2 diabetic patients and control subjects. Correlation coefficients were measured using Pearson's correlation coefficient .The criteria for statistical significance was determined at P-value less than (0.05).

Results:

Table (1) shows the clinical characteristics of the patients and controls. There were no significant differences in age and BMI means of uremic diabetic patients (group2) and control subjects of group1 ($P>0.05$). Mean duration of diabetes was (18.1±5.42, years) and mean duration of hemodialysis for the uremic patients was (4.31±2.1, months). Table (2); shows the glycemic profile in study groups. Diabetic patients in group2 (of both types) had significant higher levels of FSG, A1c when compared to healthy controls (G1), ($P<0.05$).The insulin levels were higher in diabetic patients as compared to G1, but not reached the statistical significant ($P>0.05$). Table (3); shows kidney function tests in study groups. Diabetic patients of G2 had higher mean levels of urea, creatinine, as compared to healthy controls of G1 ($P<0.05$). On the other hand estimated GFR levels were lower when compared to control subjects of G1 ($P<0.05$).While there were no significant differences between type1 and 2 diabetes in mean levels of urea, creatinine and eGFR ($P>0.05$). Blood film revealed that diabetic patients with renal failure (G2) were diagnosed to have anemia characterized by normochromic red blood cells (RBCs) with anisocytosis and anisopoikilolytosis (general term indicating an increased variation in the shape of RBCs) , this is found in a variety of anemias and hemolytic state. Table (4), represents hemoglobin level and hematocrit in study groups. Both types of diabetes in group2 had significantly reduced levels of Hb and hematocrit (Hct) when compared to G1 ($P<0.05$).No statistical significance were found in comparison between type1 and type2 of G2 ($P>0.05$). Table (5), demonstrates serum levels of EPO, ADPN and Leptin levels in study groups. Levels of EPO were higher in G2 than healthy controls of G1 ($P < 0.05$), except for type1 diabetes, which not reached the statistical significance ($P > 0.05$). Similar results were found in comparing mean levels of ADPN in both types of diabetes of G2 and healthy controls in G1 ($P < 0.05$). While no statistical difference in levels of

ADPN between type1and type2 diabetes (P > 0.05). On the other hand type2 diabetic patients had significantly increased mean levels of serum leptin than type1 diabetic patients of group2 and group1subjects (P < 0.05). Also there was statistical difference in mean level of leptin between type1 and type2 diabetes (P < 0.05).

Table (1): Clinical characteristics of diabetic, and control subjects.

	Control subjects G1	Uremic diabetic patients G2	P
Number of subjects (N)	30	30	—
*Age (years)	47.1±12.12	47.87±14.52	NS
Sex (male/female)	15/15	20/10	—
*BMI (Kg/m^2)	27.0±2.85	26.80±3.89	NS
*Duration of diabetes (years)	—	18.1±5.42	—
*Duration of hemodialysis(months)	—	4.31±2.1	—

***Data are (Mean±SD), N= no. of subjects in group, G1: group 1, G2: group 2, NS: not significant**

Table (2): Glycemic Profile in the study groups.

Analyte	G1 N=30	G2 N=30		Multiple Comparison LSD
		Type 1 N=9	Type 2 N=21	
FSG (mg/dl)	99.25± 17.19	262± 136.8	130.9± 56.63	G1vs. G2(T1) P=0.0001 G1 vs. G2 (T2) P=0.034 G2 (T1) vs. G2(T2) P=0.0001
A1c %	5.29± 0.88	9.45± 0.52	7.22± 1.44	G1 vs. G2 (T1) P=0.0001 G1 vs. G2 (T2) P=0.0001 G2 (T1) vs. G2 (T2) P=0.001
Insulin (µIU/ml)	11.24± 3.21	20.18± 12.71	12.77± 6.43	G1 vs. G2 (T1) NS G1 vs. G2 (T2) NS G2 (T1) vs. G2 (T2) NS

***Data are (Mean±SD), N=no. of subjects in group, G1: group 1, G2: group 2, NS: not significant. G2 (T1): type 1 diabetes of group 2, G2 (T2): type 2 diabetes of group 2, LSD: least significant difference test.**

Table (3): Kidney Function Tests in the study groups.

Analyte	G1 N=30	G2 N=30		Multiple Comparison LSD
		Type1 N=9	Type2 N=21	
Urea (mg/dl)	32.25± 9.09	151.1± 31.04	167.1± 50.8	G1 vs. G2 (T1) P=0.000 G1 vs. G2 (T2) P=0.000 G2(T1) vs. G2 (T2) NS
Creatinine (mg/dl)	0.67± 0.09	9.91± 4.11	8.26± 3.72	G1 vs. G2 (T1) P=0.000 G1 vs. G2 (T2) P=0.008 G2 (T1) vs. G2 (T2) NS
Uric Acid (mg/dl)	5.77± 1.74	10.65± 2.48	13.65± 4.38	G1 vs. G2 (T1) P=0.000 G1 vs. G2 (T2) P=0.000 G2 (T1) vs. G2(T2) P=0.011
eGFR (ml/min/1.73m^2)	123.34± 27.44	10.97± 12.06	8.61± 5.01	G1 vs. G2 (T1) P=0.000 G1 vs. G2 (T2) P=0.000 G2 (T1) vs. G2 (T2) NS

Table (4): Hemoglobin levels and Hematocrit in the study groups.

Analyte	G1 N=30	G2 N=30		Multiple Comparison LSD
		Type 1 N=9	Type 2 N=21	
Hb (g/dl)	13.17± 0.87	7.78± 1.24	8.03± 2.57	G1 vs. G2 (T1) P=0.000 G1 vs. G2 (T2) P=0.000 G2 (T1) vs. G2 (T2) NS
Hematocrit (%)	39.77± 4.66	25± 3.22	28.29± 7.46	G1 vs. G2 (T1) P=0.001 G1 vs. G2 (T2) P=0.000 G2 (T1) vs. G2 (T2) NS

Table (5): EPO, ADPN and Leptin levels in the study groups

Analyte	G1 N=30	G2 N=30		Multiple Comparison LSD
		Type1 N=9	Type2 N=21	
EPO (mU/ml)	34.35± 7.48	35.15± 5.57	51.61± 7.51	G1 vs. G2 (T1) NS G1 vs. G2 (T2) P=0.03 G2 (T1) vs. G2 (T2) P=0.039
ADPN (µg/ml)	9.14± 1.93	60.91± 17.15	58.72± 14.54	G1 vs. G2 (T1) P=0.027 G1 vs. G2 (T2) P=0.000 G2 (T1) vs. G2 (T2) NS
Leptin (ng/ml)	10.19± 5.97	5.75± 3.30	20.9± 4.06	G1 vs. G2 (T1) P=0.000 G1 vs. G2 (T2) P=0.04 G2 (T1) vs. G2 (T2) P=0.01

Many correlations were observed in the study groups. In group1; FSG was associated positively to age (r =0.386, P=0.039), BMI (r =0.380, P=0.042), A1c (r =0.506, P=0.032) and negatively associated to EPO (r= -0.557, P=0.002). Also ADPN was negatively correlated to BMI (r= -0.572, P=0.001). On the other hand A1c correlated positively with insulin (r = 0.590, P =0.01) and negatively with leptin (r = -0.450, P=0.016). Hb had a significant positive correlation with Hct (r =0.896, P=0.000), and eGFR (r =0.532, P=0.003). In addition eGFR correlated negatively with uric acid (r= -0.582, P=0.001), creatinine (r= -0.690, P=0.000) and ADPN (r= -0.507, P=0.007). Moreover creatinine was negatively correlated to Hb (r= -0.422, P=0.023) and Hct (r= -0.507, P=0.007). In group2 ADPN was negatively correlated to age (r = -0.482, P=0.023), BMI(r= -0.475, P= 0.025). FSG was positively associated with Alc (r=0.617, P=0.002), Insulin (r=0.553, P=0.008). eGFR was negatively related to creatinine (r= -0.74, P=0.000), urea (r = -0.65, P=0.000). Hb was positively associated with Hct (r =0.695, P=0.038). In addition EPO was positively related to Hct (r=0.788, P=0.035, figure (1)), leptin (r =0.44, P=0.046). ADPN was positively associated with urea (r =0.394, P=0.07), and negatively associated with leptin (r= -0.464, P=0.034, figure (2)) and Hb (r= -0.566, P=0.011, figure (3)).

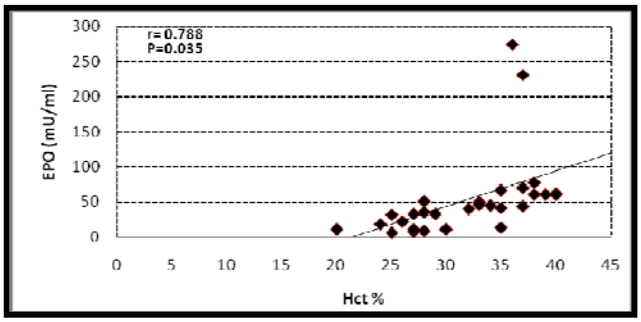

Figure (1): Correlation between EPO and Hct in G2.

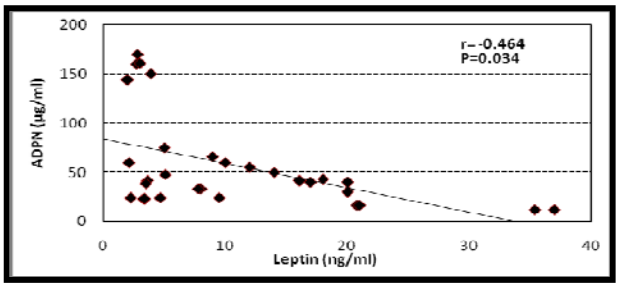

Figure (2): Correlation between ADPN and Leptin in G2

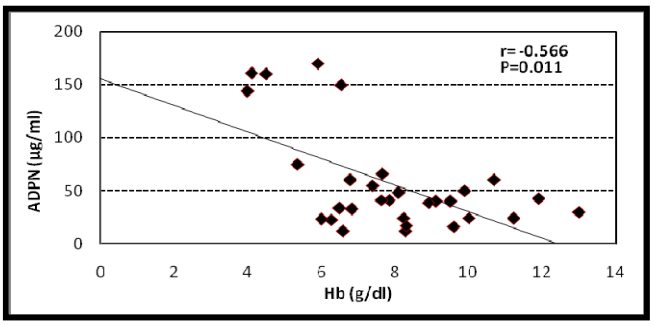

Figure (3): correlation between ADPN and Hb in G2.

Discussion:

The present study has shown that adipocytokines like ADPN and leptin have an association with anemia in diabetic patients with renal failure. These adipocytokines were found to have a vital role in diabetes and represent a significant link between adipose tissue and insulin sensitivity, beside to their role in hematopoiesis [12]. The presence of Chronic Kidney diseases (CKD) and diabetes together represent the most important aetiopathogenic combination for the development of anemia[1, 2, 4, 3and 5]. The results of the present study coincide with the results of Thomas *et al.* [1], Hamid [4], Zoccali *etal.* [13], and Aso *et al*. [14]. The reason why EPO concentration failed to increase in response to anemia has not been clarified [2]. Potential Factors include a reduced number of specific EPO-synthesizing interstitial cells and disruption of interstitial anatomy, which interferes with oxygen sensing through hypoxia-inducible transcription factor 1α (HIF-1α) [2]. A study by Inomata *et al.* [15] suggested that structural renal abnormalities may play a role in the etiology of anemia in diabetes. In this study, the diabetic patients with renal diseases showed lower EPO at baseline. An inhibitory effect of pro-inflammatory cytokines and advanced glycosylation end-products (AGEs) has also been proposed as an explanation for low EPO concentration. Other possibilities include modification of the EPO receptors by glycation or damaged to erythroid precursor cells by hyperglycemia [2]. The negative association between EPO and FSG in the present study may explain a possible effect of hyperglycemia to antagonize the increase in EPO concentration in uremic diabetic patients and confirm the previous results. The kidney is the principle site of leptin elimination, which in part explains why levels of this adipocytokine are increased among patients with renal failure. Leptin levels are related to insulin among dialysis patients [13]. Although a correlation between leptin and insulin is not found in the present study, but a negative correlation between A1c and leptin and this was in line with the data from previous study [16]. These results suggest that A1c may be a factor to influence serum leptin levels and that hyperglycemia for a long period or poorly controlled diabetes may

reduce leptin levels also directly increase insulin resistance and thereby worsening the condition. According to many studies, A1c is the best criterion for the control of diabetes and for preventing diabetes complications [16, 17]. Mean levels of serum insulin were insignificantly elevated in uremic diabetic patients as compared to healthy subjects in the present study. This may be due to the small size of samples that was divided into two types (type1 and type2 diabetes). It should be taking in account that typ1 diabetes has the higher mean level of insulin. Although the samples were taken in fasting state but the effects of treatment of synthetic insulin (the long acting treatment) are persistent in some patients. Interestingly, there is a positive correlation between EPO and leptin, because EPO treatment induces a significant decline of leptiemia among hemodialysis patients [13]. These cytokines seem to work in harmony and when EPO fails to increase in response to lower levels of Hb, leptin will act instead and vies versa as discussed by Bennet *et al.* [18]. Leptin itself appears to enhance proliferation of hemopoietic cells *in vitro*, particularly in combination with other cytokines and may augment some mature hemopoietic cells functions [18, 19]. Adiponectin is a novel, collagen-like, protein of a collection family that is mainly produced in adipocytes. Although adipose tissue is the only source of ADPN and the relationship of this protein to fat and body mass is a negative correlation [7], and this was found in the present study. Serum concentrations of ADPN are markedly increased among dialysis patients [14]. The positive correlation between ADPN and urea in the present study may explain in part the increase in ADPN levels in the study G2. Although ADPN and leptin were both increased among dialysis patients, the two hormones were negatively correlated. This phenomenon suggests that, in addition to the renal failure, other factors play a role (perhaps an important one) in the regulation of the plasma concentrations of these hormones in uremia. A previous study elegantly demonstrated that leptin behaves as a negative acute phase reactant among dialysis patients [13]. Increasing ADPN will be (act as anti-inflammatory protein) which is an appropriate adaptation to limit the deleterious effects of acute phase reaction set motion in the CRF [12]. Studies were conducted by Aso *et al.* [14], in diabetic patients with CKD and by Matsubara *et al.* [10], in anemic and non-anemic women to clarify the association between plasma ADPN concentration with red blood cells (RBCs), Hb and Hct. Plasma ADPN levels were increased in anemic compared to non-anemic subjects and were negatively associated with RBCs, Hb, and Hct, which were in line with the present study data (a negative correlation between ADPN and Hb). These results indicate the possibility that increased ADPN may contribute to the suppressive of bone marrow function *in vivo*. Combined with leptin data, adipocytes derived proteins were related to hemopoiesis; therefore it has the possible existence of adipose tissue/ bone marrow function linkage more clearly [10]. A study looked at type1 diabetics in relation to leptin and adiponectin levels revealed that type1diabetics had higher ADPN and lower leptin [20].Other studies reported higher leptin and lower ADPN levels in type2 diabetics, [7,8] these findings were found in the present study study. ADPN levels in type2 diabetes decreases, which worsen insulin sensitivity. Furthermore, prior to a decrease of ADPN levels, leptin increases and the cause for this increase is leptin resistance just like insulin resistance. The study of El-Maksod *et al.* [20] explained high ADPN levels in type1 diabetes is due to attempt of the fatty tissue to compensate for a lack of intracellular energy .With the lack of insulin , less glucose is translocated into the cellular compartments. Thus, ADPN levels would be elevated to increase insulin sensitivity of what insulin is available [20]. The prediction equation for estimation of the GFR was developed and based on the data derived from the Modification of Diet in Renal Disease (MDRD) study in patients with renal dysfunction to generate sufficiently precise, unbiased and accurate estimates of GFR to be clinically useful for evaluating kidney function [11, 21].Urea and creatinine considered as Initial diagnosis of acute or chronic kidney disease, whereas eGFR estimate renal function and use as monitoring test too [21]. A study conducted by Rosolowsky *et al.* [22] on serum uric acid levels, revealed that early renal function decline begins before the onset of proteinuria in patients with diabetes [22].The negative correlation between uric acid and eGFR in healthy subjects were in agreement with the previous results of [22].Serum uric acid can function as a proinflammatory molecule with the capacity to act as both a pro-oxidant and an antioxidant [23, 24]. In patients initiating dialysis, approximately 50% have hyperuricemia; therefore, CKD may be more likely a cause of hyperuricemia than the reverse [25].

Conclusions:

The present study demonstrates that Anemia is a common accompaniment to diabetes, particularly in those with renal failure or under hemodialysis .Adipocytokines like leptin and adiponectin may involved with anemia in these patients. Leptin in the present study was positively associated with EPO levels the hormone that stimulate hemopoiesis. On the other hand, ADPN was negatively associated with Hb. These increased levels of ADPN may antagonize EPO and leptin to increase the hemopoiesis rate and Hb levels in uremic diabetic patients in addition to other factors involved with diabetes.

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