Evaluation of The Erythropoietin Responsiveness to Anemia in Type I Diabetic Children

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Backgrounds and aim of the work: Circulating erythropoietin (EPO) levels increase during hypoglycemia and may represent protective hormonal counter-regulatory responses. The aim of this research was to evaluate the EPO responsiveness to anemia in type 1 diabetic children. **Subjects & methods:** Forty patients (20F/20 M) with type 1 DM aged from 8-18 years with history of diabetes more than 5 years.They matched with age, BMI and gender healthy 20 subjects (10 F/10 M) and 20 anemic non diabetic patients(10 F/10 M) were recruited for this study. All groups were subjected to estimation of Fasting Blood Glucose, serum EPO, ferritin, Total Iron Binding Capacity (TIBC), iron as well as Hemoglobin level, RBCs count and HbA1c in addition to micro-albumin/Creatinine ratio. **Results:** Fasting Blood Glucose concentrations showed increase significantly in patients with type 1DM than in the healthy controls and anaemic non diabetic group (P**<0.001)** Serum EPO levels were significantly higher in patients with type 1DM than in the healthy controls (P**<0.05)** While serum EPO levels showed statistically highly significant difference between diabetics and anemic non diabetic group (49.85±24.91 & 212.54±65.31respectively P**<0.001**). Diabetic patients had lower Hb levels than healthy one ($p<0.05$) and non significant difference than anemic patients.Also, diabetic patients showed significant increase in ferritin and iron levels and significant decrease in TIBC levels than anemic patients group. There were statistically significant differences between diabetics with positive microalbuminuria (n=12) and diabetics with normoalbuminuria (n=28) as regarding HbA1c concentrations, Hb concentrations and a highly significant difference as regarding serum EPO levels. EPO levels showed significant positive correlation with HbA1c concentrations and highly significant positive correlation with microalbuminuria in diabetic group and showed inverse correlation with Hb levels in the same group. [El Hefnawy, H., Haider, N., Emara, I. and Ghanem, A. **Evaluation of The Erythropoietin Responsiveness to Anemia in Type 1 Diabetic Children.** *Nat Sci* 2013;11(6):146-153]. (ISSN: 1545-0740). http://www.sciencepub.net/nature. 17

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1. Introduction

The triad of diabetes mellitus, anemia, and chronic kidney disease (CKD) define a group of patients at high risk for death and cardiovascular complications. The approval of epoetin alfa in 1989 transformed the treatment of anemia in patients with CKD. However, evidence has emerged from randomized controlled trials that correcting anemia with erythropoiesis-stimulating agents in CKD patients is associated with increased risk ***(Singh,. 2010)***. A common feature among all forms of diabetes mellitus is a functional β-cell mass insufficient to maintain euglycemia; therefore, the promotion of β-cell growth and survival is a fundamental goal for diabetes prevention and treatment. Evidence has suggested that erythropoietin (EPO) exerts cytoprotective effects on non erythroid cells. However, the influence of EPO on pancreatic β cells and diabetes has not been evaluated to date ***(Diana Choi et al,.2010).***It was demonstrated that in patients with type 1 diabetes that the concentration of EPO increases during hypoglycemia, and that low baseline EPO levels may be associated with more pronounced cerebral dysfunction during hypoglycemia (nadir plasma glucose concentration 2.2 mmol/l) than higher levels of EPO ***(Kristensen,.et al.,2009)*** Correction of anemia to normal hemoglobin concentrations is associated with significant adverse cardiovascular outcomes and is not recommended, escalating doses of erythropoiesis-stimulating agents should be avoided. The treatment of anemia in people with diabetes and chronic kidney disease should begin with optimisation of iron stores. An aspirational hemoglobin concentration range of 10-12 g/dl with anemia management, balances proven benefits of anemia treatment with potential cardiovascular risk ***(Stevens., 2012).***

EPO is produced in the kidney and the liver and stimulates the differentiation and proliferation of erythroid progenitors. The subsequent synthesis of recombinant human EPO markedly changed the management of ESRD-related anemia. Other findings that erythropoiesis-stimulating agents (ESAs) have not improved cardiovascular and renal outcomes in patients with non–end stage CKD ***(Dru¨eke,. et al.,2006, Singh, et al.2006 and Pfeffer, et al.,2009)*** have raised questions about the timing and degree of

EPO deficiency in renal anemia ***(Fishbane, et al., 2010)***. In diabetic patients with CKD, elevated endogenous EPO levels were predictive for mortality and were related mainly to markers of inflammation, independent of kidney function, and despite low hemoglobin levels. Understanding the phenomenon of EPO resistance and iron dysregulation caused by inflammation is crucial for effective and safe treatment of anemia in patients with CKD ***(Martin Wagner, et al., 2011)***.

Normochromic normocytic anemia can occur in various chronic diseases, and it appears that dysregulation of iron homeostasis and inflammatory processes act as the main mediators ***(Ezekowitz, et al 2003, Ganz.,2007 and Weiss, Goodnough., 2005)***. Similarly, anemia in diabetic CKD is likely multifactorial, involving EPO deficiency as well as iron dysregulation and inflammation ***(Menon, et al., 2003, Soriano, et al., 2007 and Zimmermann, et al 1999)***.Absolute EPO deficiency can be caused by diminished EPO production as well as by EPO-sensing errors (***Ferrucci, et al., 2007 and McGill, Bell., 2006);*** however, other data also describe functional EPO deficiency or EPO resistance, which is characterized by a low hemoglobin despite EPO levels that are within the "normal" range ***(Thomas, et al., 2006).***

The purpose of this study was to evaluate the EPO responsiveness to anemia in type 1 diabetic children. Also, We aimed to identify factors associated with EPO levels, with a particular focus on positive microalbuminuria, as well as to investigate the relationship between EPO levels and all-cause mortality risk.

2. Subjects and Methods

The study was performed on forty outpatients with type 1 diabetic children aged from 8-18 years with history of diabetes more than 5 years. They were randomly collected from the outpatient pediatric clinics of National Institute for Diabetes & Endocrinology (NIDE), Cairo, Egypt. Type 1 DM was diagnosed according to the Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (2006). They were 20 females and 20 males. All of them were screened for anemia, with hemoglobin (Hb) levels < 11 g/dl. We chose this Hb cut-off value because a significant EPO response is present only below this level ***(Spivak, 1993)***. Those with identified causes (e.g. ferritin, B12, folate deficiencies, thyroid dysfunction, liver diseases, malignancies, advanced nephropathy and hemoglobinopathies) were excluded.The control group consists of 20 healthy non diabetic subjects, had no recognizable diseases and clinically free from any abnormality. Other group consists of 20 subjects anemic (hypochromic microcytic iron deficiency anemia), non diabetic children with same range of Hb concentration as the study group. They were comparable to study group as regarding age, sex, BMI and socioeconomic classes. data was recorded for each subject using self-made questionnaire. Approval had been taken from the research ethics committee of General Organization of Teaching Hospitals and Institutes. An informed consent was obtained from all patients and healthy subjects.

Blood samples were collected into vacutainer tubes without additive after 12 h overnight fasting from healthy subjects and diabetic patients, Blood was centrifuged at 3000 rpm for 10 minutes. Serum was rapidly separated; subdivided into aliquots One aliquot should be used fresh for determination of Fasting Blood Glucose at once by Hexokinase methods according to the method of ***(Kunst A,et al 1983***). Iron,TIBC were determined colorimetrically using according to the methods of ***(Ruutu, 1975),(Ceriotti and Ceriotti,1980)*** respectively and Serum creatinine was measured by colorimetric method according to ***(Vasiliades, 1976)***., and the second aliquot was preserved at -80ºC. to estimate Ferritin concentration was measured using commercially available enzyme- linked immunosorbent assay (ELISA) kits Immunospec coroperation (Catalog No 29-013) according to the method of (***White et al.1986)*** and EPO level was measured using commercially available enzyme- linked immunosorbent assay (ELISA) kits from DRG International Inc., USA (Catalog No EIA -3646) according to the method of ***(Spivak, 1995)***. Another part of collected blood was taken on EDTA for determination of HbA1c using ion-exchange high-performance liquid chromatography (HPLC) with Bio-Rad Variant Hemoglobin Testing System (Bio-Rad Laboratories, USA) according to method of ***(Lahousen et al; 2002),*** Hb level and RBCs count were measured using *ADVIA 120* (Bayer, Germany) automated system. Fresh morning urine samples was collected from each subject into sterile cups and used for determination of microalbumin by immuno-turbidimetric method according to ***(Gentilini et al 2005).*** and urinary creatinine according to***(Vasiliades 1976)***. Estimation of albumin to creatinine ratio (ACR) according to (***Justesen et al 2006)***. BMI was calculated at baseline of study as weight Kg/height m^2 according to (***Slynkova et al. 2006).***

Statistical analysis

Data was expressed as the mean ± S.D. Statistical analysis was performed with Statistical Package for

the Social Science for Windows (SPSS, version 10.0, 1999, Chicago, IL, USA).

Differences between groups were analyzed by one-way analysis of variance (ANOVA). Post-hoc testing was performed by the Bonferroni test to compare the difference among the groups studied as reported by ***Altman (1991).***Furthermore, analysis was performed to examine the possibility for any correlation between different parameters. For clinical correlations, the correlation co-efficient was calculated using least square method.All reported P- values were two-tailed, value <0.05 was considered significant.

3. Results

Table (1) showed that there were no statistically significant differences between diabetics and two other studied groups as regarding age, sex, BMI and serum Creatinine levels.However, There was significant increase between diabetics and two other studied groups as regarding FBG,HbA1c and urinary A/C levels. There was significant decrease between diabetics and healthy control group as regarding the RBCs count (3.35±0.56 & 4.88 + 0.76). Also, there was a significant difference between diabetic group and the control group as regarding serum EPO levels(49.85±24.91 & 39.31±21.86 respectively).On the other hand, serum EPO levels showed statistically highly significant difference between diabetics and anemic non diabetic group (49.85±24.91 & 212.54±65.31 respectively) (figure-1). There was a significant increase in the levels of iron and TIBC in diabetic group than control one. Ferritin levels showed a non significant difference in diabetic group against control one but showed significant increase in diabetic group against anemic group.

Table (2) showed that there were statistically significant differences between diabetics with positive microalbuminuria (n=12) and diabetics with normoalbuminuria (n=28) as regarding HbA1c concentrations, and Hb concentrations and a highly significant difference as regarding FBG, and serum EPO levels. There was no statistically significant difference as regarding serum Creatinine levels between both groups.

Table (3) showed that there was a negative significant correlation between Hb concentration in diabetic group (mean=9.85±1.64 gm%) and serum EPO (mean=49.84+24.91 mlU/ml). There was a positive significant correlation between serum EPO levels in diabetics, and glycated HbA1c concentrations in diabetic group. While there was a highly positive correlation between mean EPO level and microalbuminuria in this group.Also there was a highly positive correlation between mean EPO level and FBG.

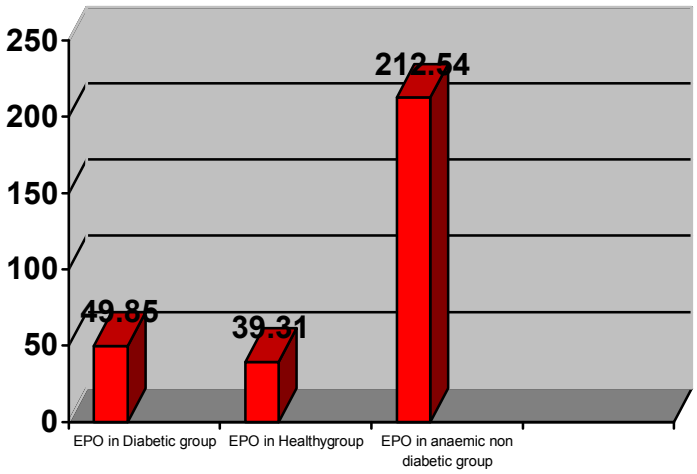

Figure (1): Comparison between EPO levels in diabetic children, healthy control group and anemic non diabetic group

Table (1): Comparison between study group (diabetics), and control groups as regarding different variables.

DATA	GROUP(1) (Diabetic) N=40		GROUP(2) Control)(N=20		GROUP(3) (Non diabetic - Anemic) N=20		P For1#2	P For1#3
	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.		
Age (6-18 years)	12.42	5.62	12.39	6.11	12.16	4.99	**>0.05**	**>0.05**
Sex (F/M)	20/20		10/10		10/10		**>0.05**	**>0.05**
BMI (kg/m2)	21.66	3.11	19.6	4.94	20.10	4.24	**>0.05**	**>0.05**
FBG(mg/dl)	258.70	24.73	73.25	5.82	81.67	8.34	**<0.001****	**<0.001****
HbA1c(%)	8.6	4.71	4.1	1.91	3.9	2.15	**<0.001****	**<0.001****
Hb (gm/dl)	9.85	1.64	13.95	2.16	10.17	1.16	**<0.05***	**>0.05**
RBCs x 10^6/cumm	3.35	0.56	4.88	0.76	3.46	0.4	**<0.05***	**>0.05**
EPO (mIU/ml)	49.85	24.91	39.31	21.68	212.54	65.31	**<0.05**	**<0.001****
Ferritin (ng/ml)	70.95	45.84	68.95	36.82	52.62	32.31	**>0.05**	**<0.001****
Iron (mg/dl)	49.34	22.19	31.51	12.95	42.34	22.12	**<0.001****	**<0.05***
TIBC (Ug/dl)	289.65	98.62	198.65	21.65	354.98	89.65	**<0.001****	**<0.001****
Urinary A/C (mg/gm cr.)	32.54	11.21	9.62	5.65	8.51	4.31	**<0.001****	**<0.001****
Creatinine (mg/dl)	0.97	0.31	0.95	0.48	0.89	0.45	**>0.05**	**>0.05**

*= significant
**= Highly significant

Table (2): Comparison between diabetics with positive microalbuminuria and diabetics with normoalbuminuria.

DATA	DIABETICS WITH POSITIVE MICROALBUMINURIA N=12		DIABETICS WITH NORMOALBUMINURIA N=28		P
	MEAN	***S.D.***	***MEAN***	***S.D.***	
FBG(mg/dl)	313.4	26.99	226.1	21.32	<0.001**
HbA1c (%)	10.20	3.11	8.40	3.97	<0.05*
Hb (gm/dl)	9.11	2.19	10.25	1.14	<0.05*
RBCs x 10^6/cumm	3.18	0.76	3.85	0.399	>0.05
EPO (mlU/ml)	36.54	15.98	98.12	38.75	<0.001**
Creatinine (mg/dl)	1.05	0.54	0.95	0.38	>0.05
A/C ratio (mg/gm cr.)	65.52	21.12	21.54	6.21	<0.001**

*= significant
**= highly significant

Table (3): Correlation between serum EPO levels to different variables.

DATA	EPO (mean=49.84+54.91 mlU/ml)	P
	r	
Hb (gm%) (mean=9.85±1.64)	-0.481	<0.05*
HbA1c (%) (mean=8.6±4.71)	0.371	<0.05*
Urinary A/C (mg/gm cr.) (mean=52.54±31.21)	0.198	<0.001**
FBG mg/dl (mean=258.7±24.73)	0.231	<0.001**

*=significant

**=highly significant

4. Discussion

Anemia is a common finding in people with diabetes and chronic kidney disease and failure of the kidney to produce erythropoietin in response to a falling hemoglobin concentration is a key component, correlating with the degree of albuminuria, renal dysfunction and iron deficiency *(**Stevens 2012)***. In this study, we examined the relation between serum EPO levels and HbA1c concentrations in diabetic group, also, its relation with microalbuminuria in this group. In our study we found that Serum EPO concentrations in diabetics with microalbuminuria was significantly lower than that in diabetics with normoalbuminuria.

In the current study, it has been demonstrated in patients with type 1 diabetes that the concentration of EPO increases during hypoglycemia, and that low baseline EPO levels may be associated with more pronounced cerebral dysfunction during hypoglycemia (nadir plasma glucose concentration 2.2 mmol/l) than higher levels of EPO ***(Kristensen. et al.,2010).***

Anemia occurs early in the course of diabetic nephropathy ***(Bosman.et al 2001)*** yet testing for anemia is not considered by NICE guidance for management of diabetes (NICE 2010), nor by the 2011 guidance issued by the American Diabetes Association (ADA) (ADA 2011). The Scottish Intercollegiate Guidelines Network (SIGN Management of Diabetes guideline is the only national guidance in diabetes management to specifically address anemia management (SIGN 2011). SIGN recommend that people with diabetes and stage 3–5 CKD have their hemoglobin checked at least annually. The anemia with EPO deficiency was described in some type 1 diabetic patients with severe symptomatic diabetic neuropathy ***(Ricerca.et al 1999 and Winkler.et al 1999,)***.EPO release is thought to be modulated by the splanchnic innervations of the kidney because renal denervation in animal modules leads to a loss of EPO production in response to hypoxic stimuli ***(Beynon.1977 and Spannhake.1977***). It has been postulated that EPO deficiency in these patients may be caused at least in part by efferent sympathetic denervation of the kidney leading to the loss of appropriate EPO production ***(Takaku.et al,1991),*** and there is some clinical and experimental evidence that this may be the cause ***(Fioretto.et al 1996).*** However, all of these autonomic neuropathy patients also had evidence of diabetic nephropathy with persistent proteinuria, although so had only microalbuminuria, because the lesion of diabetic nephropathy may involve not only the glomeruli but also the renal interstitial area ***(Bosman.et al 2001)***. It is possible that the EPO deficiency of these patients may result from the damage to the EPO-producing fibroblasts and is not a

consequence of the neuropathy itself ***(Inomata et al.1997).***

Cotroneo et al (2000) studied the clinical and biochemical characteristics of diabetic patients with anemia of uncertain cause and measured EPO concentrations in 35 diabetic subjects without significant diabetic renal disease. They found that the serum EPO concentrations of diabetic patients were significantly lower than those of non diabetic patients with similar degree of decrease in Hb concentrations. They concluded that reduced EPO responsiveness to anemia present but without advanced diabetic nephropathy and this might reflect early renal interstitial damage. ***El Hefnawy, and Anis, 2001***., studied 13 type 1 diabetic patients with Hb levels < 11 gm/dl. They stated that the majority of them who had anemia also had low EPO levels and the pathogenesis of this phenomenon is probably multifactorial that might include the autonomic neuropathy with respect to the effect of the renal damage**.**

Potential contributors to the development of anemia in diabetes revolve around either impaired production and/or response to EPO, with or without concomitant haematinic deficiency***(Stevens.,2012).***. In patients with diabetes found that EPO deficiency was present in 34% of WHO anemia, and abnormal haematinics in 40%, but anemia was unexplained in 26%. Unsurprisingly people with anemia and diabetes were more likely to be taking ACE inhibitors or angiotensin II receptor blockers however a causative role for rennin - angiotensin system blockade in the genesis of anemia in patients with diabetes has yet to be shown. In 604 patients with type 2 diabetes ***(Jones et al 2010)***. ***Thomas et al(2006)*** found that 19% (n _ 112) were anemic, EPO deficiency was present in 76% and reduced iron availability in 58%. Even in the absence of renal impairment, 32 of 45 anemic patients had an impaired EPO response and two-thirds of patients with reduced iron availability were unable to increase EPO levels above the normal range. In patients without persistent microalbuminuria, in both type 1 and type 2 diabetes, tubulo-interstitial damage, together with low levels of EPO at normal categories of renal function, has been described ***(Singh et. al., 2009).***

Khoshdel et al (2008) stated that patients with diabetes mellitus were more likely to be iron deficient, a barrier to effective rhEPO therapy. The effect of treatment on the reliability of HbA1c as an index of glycemic control must be remembered. It is proposed that anemia and its causes must be important components of care in subjects with early diabetic renal damage.

From all the above, it could be concluded that the normocytic normochromic anemia with reduced EPO responsiveness could occur early in young type 1diabetic patients even before the diagnosis of diabetic nephropathy So, it is recommended for type 1 diabetics to do regular blood counts for early detection of any decrease in Hb concentration with estimation of serum EPO responsiveness as early markers of diabetic nephropathy and neuropathy. It could be recommended also to do more studies about the effectiveness of EPO use in anemic type 1 diabetic patients even before the diagnosis of diabetic nephropathy.

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