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Prevalence of Anti-erythropoietin Antibody in Prevalent Hemodialysis Patients Receiving Erythropoietin Hormone

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ABSTRACT

Background: Anti-erythropoietin (EPO) antibodies against a recombinant form of Erythropoietin were associated with pure red cell aplasia. These antibodies probably cross-react with endogenous EPO causing severe anemia.

Objective: This study was done to estimate the prevalence of anti-EPO antibody in prevalent hemodialysis patients and its relation to anemia and EPO resistance.

Patients and Methods: This is cross-sectional study included 90 End-Stage Renal Disease (ESRD) patients who were on recombinant human erythropoietin for > 6 months with hemoglobin< 11 g/dL. Serum anti-EPO antibody was measured for all Patients that were divided into 2 groups: anti-EPO antibody positive and anti-EPO antibody negative.

Results: The prevalence of anti-EPO antibodies was 45.6% (41 patients). 58.5% (24 patients) received EPO treatment by S.C route. Anti-EPO antibody-positive patients Showed lower hemoglobin level, serum iron, and Transferrin saturation with mean ±SD values 8.60±0.99 g/dl, 52.91±16.30 mcg/dl, 22.62±8.27 % respectively also lower MCHC with median (IQR)29.7 (2.50) g/dl. On comparing it with antibody-negative patients (N=49) there were significant differences with p values (< 0.0001), (0.038), (0.034), (0.002) respectively. These patients received higher EPO doses with high erythropoietin resistance index with median (IQR) values 8000 (4000) IU/week, 10.79 (8.495) respectively. The anti-EPO antibody was negatively correlated with hemoglobin {r-0.661, p value 0.0001} and positively correlated with EPO doses, iron doses, and erythropoietin resistance index {r 0.309, p value 0.011, r 0.266, p value 0.003, r 0.417, p value 0.0001} respectively.

Conclusions: It could be concluded that anti-EPO antibodies are commonly present in prevalent hemodialysis patients and were associated with resistant anemia and higher doses of EPO therapy.

Keywords: Anti-erythropoietin, Antibody, Hemodialysis.

INTRODUCTION

Anemia is one of the most frequent early complications of chronic kidney disease (CKD)[1]. It is associated with worse quality of life, reduced exercise capacity, decreased mental agility, and increased prevalence of hospitalization and mortality [2].

The main cause is erythropoietin (EPO) deficiency due to impaired kidney function. However, other causes should be considered when the severity of anemia is inconsistent with the decrease in renal function. Treatment of anemia in CKD patients usually involves the use of recombinant human erythropoietin (rHuEPO). The main cause of rHuEPO treatment failure is the loss or low iron availability [3].

The prevalence of iron deficiency is very common in CKD, affecting as many as 50% of patients [4]. Multiple other factors have been identified that may further exacerbate anemia developing in patients with impaired renal function. Some of these factors may be important for the development of anemia (e.g., inflammatory processes or tumors), while the meaning of others is only coincidental (e.g., folic acid or drugs), or more or less hypothetical (e.g. uremic inhibitors of erythropoiesis) [5].

There is no consensus about the definition for rHuEPO resistance. It is defined as persistent anemia (hemoglobin<10–12 g/dL) or the necessity of very high doses of EPOetin alfa (300 IU/kg/week subcutaneously or 450 IU/kg/week intravenously. The evaluation of resistance is recommended if there is an increase of≥25% in erythropoietin dose or <1 g/dL gain in hemoglobin levels after 2–4 weeks of treatment[6].

One of the causes of resistance to treatment with rHuEPO in dialysis patients is anti-erythropoietin antibodies although it is well tolerated by most patients, a small number produce antibodies that can neutralize both endogenous EPO and recombinant proteins [7]. Most cases of antibody production have been associated with the formulation of EPOetin alfa when administered subcutaneously [8].

In some cases, the anti-erythropoietin (anti-EPO) antibody production can lead to the development of serious PRCA (pure red cell aplasia) and transfusion-dependent anemia[9]. Recent studies have shown that anti-EPO antibody-mediated PRCA is rare but has an important adverse effect in patients with CKD who take rHuEPO[10].

This study was done to estimate the prevalence of anti- EPO antibody in prevalent hemodialysis patients and its relation to anemia and EPO resistance.

PATIENTS AND METHODS:

This cross-sectional study included a total of 90 ESRD patients, from Suez Insurance Hospital and Ain Shams University Hospital hemodialysis units.

Patients were on HD, receiving 3 sessions per week, each session four hours using bicarbonate dialysate and heparin as an anticoagulant. Patients were on recombinant human erythropoietin (rHuEPO) > 6 months and their hemoglobin< 11 g /dL.

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Exclusion Criteria: patients with malnourishment, chronic liver disease, hypothyroidism, ongoing active autoimmune disease, active infection until cured, on steroid therapy, with bleeding or hemolysis, and elderly.

Serum anti-EPO antibody was measured for all Patients that were divided into 2 groups: anti-EPO antibody positive and anti-EPO antibody-negative patients.

All patients were subjected to detailed medical history taking, demographic data, routine laboratory investigations, C – reactive protein, TSH, calculation of Erythropoietin Resistant Index (ERI) = (EPO/wt.) /Hb). ERI is defined as the average weekly erythropoietin (EPO) dose [IU] per kg body weight (wt.) per average hemoglobin [g/dl]) (Hgb), over 3 months [11].

Serum anti-Erythropoietin antibodies measurement:

Anti-EPO antibody was measured by ELISA Kit based on double antibody sandwich Enzyme-linked Immune-Sorbent assay technique and anti-erythropoietin antibody titre < 35 mU/ml was considered negative.

***Ethical Consideration*:**

An approval of the study was obtained from Ain Shams University Academic and Ethical Committee. Written informed consent of all the subjects was obtained. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis

Data were collected, revised, coded, and entered into the statistical package for the social science, version 20 (SPSS Inc., Chicago, Illinois, USA). The qualitative data were presented as number and percentage, whereas quantitative data were presented as mean with SD or median with interquartile ranges (IQR) for nonparametric data (duration of hemodialysis, TLC, MCH, MCHC, Ferritin, ERI, iron dose, EPO dose, CRP, PTH). Comparison between two groups with qualitative data was done by using the $\chi 2$ test. Comparison between two groups with quantitative data was done by two-tailed independent t-test when the distribution of the data was found parametric. Mann–Whitney test was used with the nonparametric data. Spearman correlation coefficients were used to assess the correlations. P value < 0.05 was considered significant.

RESULTS

Table 1 shows the demographic and laboratory data of the studied groups. The main causes of end stage renal disease in the study were hypertension 54.4% (49 patients) followed by unknown 13.3% (12 patients) and DM 11.1% (10 patients) polycystic kidney disease 8.9% (8 patients) other causes 12.2% (11patients). Anti-EPO antibody-positive patients (N=41) 48.8 5% (20 patients) were females and had lower MCHC and serum PTH with median (IQR) values of 29.7 (2.50) g/dl, 276 (251.549) pg/ml respectively. On comparing it with antibody-negative patients there was a significant statistical difference with p values 0.04, 0.002, 0.037 respectively. 58.5% (24 patients) received EPO treatment by S.C route and it has a significant relation to anti-EPO antibodies development compared to antibody-negative group 28.6% (14 patients) p-value (0.001*). Anti-EPO antibody-positive patients (N=41) showed lower hemoglobin level, serum iron, and transferrin saturation with mean ±SD values 8.60±0.99 g/dl, 52.91±16.30 mcg/dl, 22.62±8.27%, respectively on comparing it with antibody-negative patients there was a significant statistical difference with p values < 0.0001, 0.038, 0.034 respectively (figure 1). The patients received higher EPO doses with high erythropoietin resistance index with median (IQR) 8000 (4000) IU/week, 10.79 (8.495) respectively. On comparing it with antibody-negative patients p values were (0.006), (0.0001) respectively (Table 1). There were significant correlations. Anti-erythropoietin antibody was negatively correlated with haemoglobin level, transferrin saturation and PTH {r-0.661, p value (0.0001)},{r-0.220, p value (0.038)},{r-0.259 , p value (0.014)} respectively and positively correlated with MCV, EPO doses, iron doses, and erythropoietin resistance index {r 0.227 , p value (0.031)}, {r 0.309, p value (0.011)},{r 0.266, p value (0.003)}, {r 0.417, p value (0.0001)} respectively (Table 2 & Figures 2, 3).

Table (1): The demographic & laboratory parameters of the studied groups.

	Anti –EPO AB Positive N=41	Anti-EPO AB Negative N=49	P-value
Age (years)	51.51±11.71	49.80±11.46	0.048
Females n (%)	20 (48.8%)	14 (28.6%)	0.04*
SC EPO n (%)	24(58.5%)	14(28.6%)	0.001*
BMI (kg/m^2)	27.25± 3.91	27.82±3.94	0.493
Urea Reduction Ratio (URR)	64.16±8.33	66.11±10.43	0.337
Ca (mg/dl)	8.60±0.94	8.51±0.87	0.656
PO4 (mg/dl)	5.20±1.78	4.64±1.20	0.096
S. Albumin g/dl	3.50±0.34	3.64±0.43	0.080
Platelet count (mcL)	235.24±52.46	223.14±42.91	0.408
MCV (fl)	92.10±12.32	89.15±7.41	0.164
TSH (IU/ml)	2.61±0.84	2.54±0.91	0.707
Hb (g/dl)	8.60±0.99	10.63±1.29	0.0001*
S. iron (mcg/dL)	52.91±6.30	65.10±3.84	0.038*
TIBC (mg/day)	244.41±54.68	232.84±59.12	0.341
Transferring saturation	22.62±5.27	28.06±4.29	0.034*
On Iron therapy (%)	18(43.9%)	13 (26.5%)	0.05*
No Iron Therapy (%)	23(56.1%)	36(73.5%)	
Duration of HD/month Median (IQR)	48 (53)	52 (80.5)	0.258
TLC (*10^3/µl)	7.43 ±1.43	7.21±1.31	0.857
MCH (pg)	27 ±5.31	27.5 ±6.32	0.542
MCHC (g/dl)	29.7 ±6.38	31.7 ±7.11	0.002*
CRP (mg/L)	1±0.32	1±0.31	0.653
PTH (pg/mL)	276 ±59.81	364 ±70.91	0.037*
S. ferritin (ng/mL)	441.5 ±90.51	429.5 ±88.91	0.617
Dose of iron (week) Median (IQR)	100 (100)	50 (50)	0.230
Dose of EPO. IU (week) Median (IQR)	8000 (4000)	4000 (4800)	0.006*
EPO resistance index	10.79 ±3.91	5.08 ± 1.42	0.0001.*

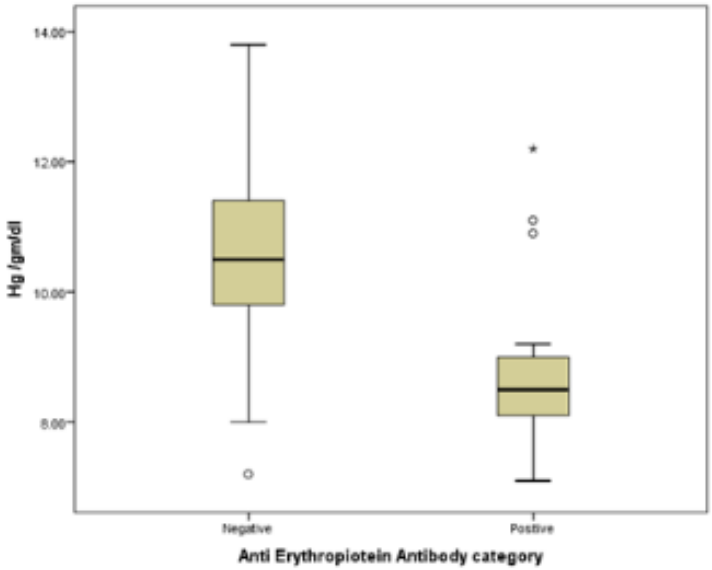

Fig. (1): Box plot for the level of Hb (mg/dl) in patients with positive and negative anti-erythropoietin antibodies.

Table (2): Correlation between anti-erythropoietin antibody and different studied variables.

Anti-Erythropoietin Antibody	Correlation coefficient	P value	Sig
S. Albumin (g/dl)	-.187	0.078	NS
Corrected ca (mg /dl)	-.033	0.757	NS
Po4 (mg/dl)	.167	0.115	NS
TLC (*10^3 /µl)	.143	0.178	NS
Platelet count (mcL)	.069	0.520	NS
CRP (mg/l)	-.050	0.640	NS
TSH (IU/ml)	.143	0.180	NS
Number of sessions (week)	-.038	0.720	NS
PTH (ng/L)	-.259-*	0.014	S
Hb (g/dl)	-.661-**	0.0001	S
MCV (fl)	.227*	0.031	S
MCH (pg)	.188	0.076	NS
MCHC (g/dl)	.176	0.097	NS
Serum iron (mcg/dL)	-.190	0.074	NS
TIBC (mg/day)	.142	0.183	NS
Serum ferritin (ng/mL)	-.086	0.418	NS
Transferrin saturation %	-.220-*	0.038	S
EPO IU dose (week)	.309**	0.003	S
Iron dose (week)	.266*	0.011	S
Erythropoietin resistance index	.417**	0.0001	S

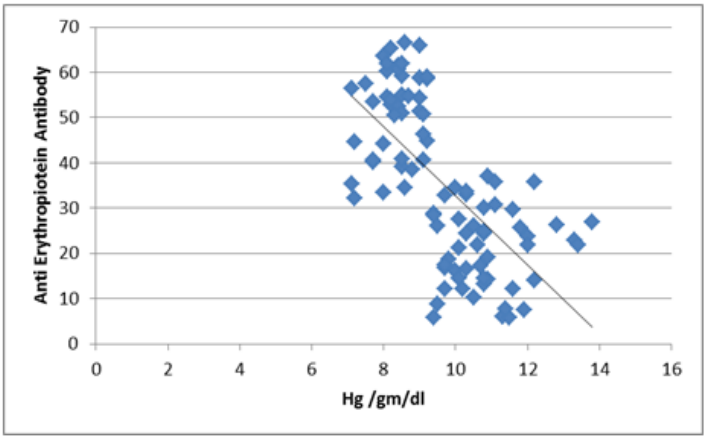

Fig. (2): Correlation between anti-erythropoietin antibody and haemoglobin.

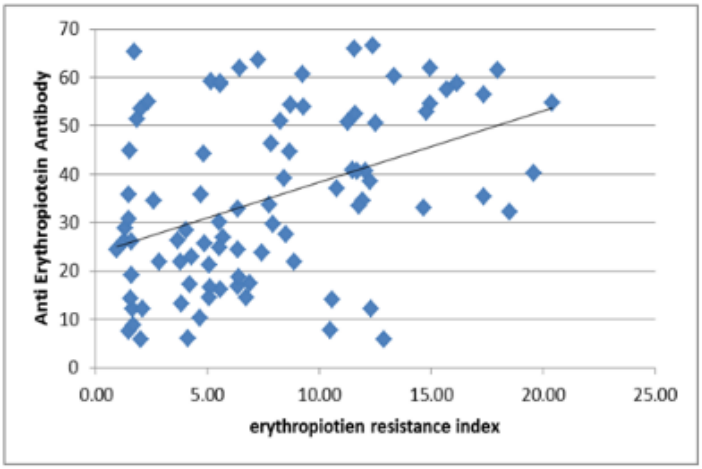

Fig. (3): Correlation between anti-erythropoietin antibody and erythropoietin resistance index.

DISCUSSION

Anti- EPO antibodies can neutralize either endogenous EPO or recombinant proteins that cause EPO resistance. It has an important adverse effect that lead to the development of serious PRCA in patients with CKD [9]. The purpose of this study was to estimate the prevalence of anti- EPO antibody in prevalent hemodialysis patients and its relation to anemia and EPO resistance. The prevalence of anti-erythropoietin anti-body was 45.6% (41 patients). This result is consistent with **Castelli *et al.*** [12] who suggested that up to 67% of ESRD patients treated with rHuEPO developed anti erythropoietin antibodies**.** Also **Puri *et al.*** [13] showed that up to 69% of patients tested positive and 61% showing weak binding demonstrates that the assay is highly sensitive and can detect even the low affinity circulating antibodies.

Data obtained from our study showed that 58.5% (24 patients) received EPO treatment by S.C route and it has a significant relation to anti-EPO antibody development. This is explained by **Shimizu *et al.*** [14] who observed that subcutaneous administration of EPOetin is an important risk factor for immunogenicity while intravenous use of proteins was generally associated with the lowest risk of immunogenicity.

In our study anti-EPO, antibody-positive patients Showed lower hemoglobin level, MCHC, serum iron, transferrin saturation, and PTH on comparing it with antibody-negative patients. These results were different from **Alqahwaji *et al.*** [15] that showed no significant difference between the studied groups regarding serum iron and TSAT either in positive or negative ant-EPO antibodies groups. Also **De Vecchi *et al.*** [16] demonstrated that PTH levels of 300, 600, and 900 pg/mL were associated with approximately 90%, 79%, and 67% of the maximum response to treatment with rHuEPO, respectively. So Patients with controlled PTH had better response with erythropoietin therapy if no anti-EPO antibody or other factors of anemia resistance. The effect of very high PTH on RBC has different mechanisms that will appear earlier before the development of anti-EPO antibodies effect. The severity of secondary hyperparathyroidism and the extent of bone marrow fibrosis increase the dose of EPO needed to achieve an adequate haematocrit [17]. Osmotic fragility was examined by **Bogin *et al.*** [18]. They demonstrated that PTH increases the osmotic fragility of RBC through enhanced calcium entry into RBC. A subsequent study by **Akmal *et al.*** [19] further showed that 51Cr-labeled RBC survival was shortened in 5/6 nephrectomized dogs, but the reduced RBC survival was attenuated and normalized in uremic dogs with parathyroidectomy.

We found that Antibody positive patients received higher EPO doses with a high erythropoietin resistance index compared with antibody-negative patients. This is agreed with **Kharagjitsingh *et al.*** [20] who found that higher doses of EPO were significantly associated with anti-EPO antibodies.

There were significant correlations. Anti-erythropoietin antibody was negatively correlated with Hemoglobin level, transferrin saturation, and PTH respectively. This confirms **Zhi-yuan *et al.*** [21] results that Hb is negatively correlated with anti-erythropoietin antibody and PTH.

CONCLUSION

It could be concluded that anti-EPO antibodies are commonly present in prevalent hemodialysis patients and one of the causes of anemia resistance. It is associated with receiving higher doses of EPO therapies.

Limitation of this study was the small number of patients. Further studies are needed on large number of patients discussing the protocols of anemia management in anti-EPO antibodies positive patients.

Conflicts of Interest: The authors declare that there are no conflicts of interest regarding the publication of this paper.

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