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ORIGINAL ARTICLE

Detection of Anti-Erythropoietin Antibodies in Patients with Chronic Renal Failure Undergoing Hemodialysis

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ABSTRACT

Background: Erythropoietin, a hormone produced by the kidney, activates the bone marrow upon demand. Recombinant erythropoietin can be subcutaneously injected to maintain red blood cell counts in chronic renal failure patients. Some time being recognized as foreign antigens, the patient occasionally produces antibodies against erythropoietin. Objective of current study was to investigate the existence of Anti-Erythropoietin Antibodies in Chronic Renal Failure **(**CRF) affected individuals during hemodialysis.

Methods: The current study was performed at the Pathology Department, Khyber Teaching Hospital, Peshawar, Pakistan. After the assent of ethical committee, data collection was initiated to carry out the study. All the patients filled the informed consent proforma. The blood sample was collected in two EDTA and Gel tubes for complete blood count and for anti-EPO-antibodies respectively. ELISA tests for anti-EPO antibodies were performed on all patients who were classified into two groups based on whether antibodies were present or not. The data was analyzed using SPSS version 25.0.

Results: In this study, 150 patients suffering from Chronic Kidney Disease on dialysis were included in which 79 (52.7%) were males. Moreover, age-wise group 41-60 years (n=93, 62%) were more suffering as compared to age group 21-40 years (n=24, 16%). In our study, hypertension was found to be a leading cause of death for 65(43.3%), diabetes 32(21.3%), hypertension and diabetes mellitus 10(6.7%), and miscellaneous accounted for 28.7%. There were 56(37.3%) patients who received dialysis twice a week, and 94 patients (62.6%) received it three times a week. Patients had transfusion history were 136(90.7%), while 92 (61.3%) patients were found to have anti-EPO antibodies. **Conclusion:** Anti-EPO antibodies are frequently demonstrated in patients with CKD undergoing hemodialysis. Due to the lack of correlation between antibody levels and Hb or reticulocyte counts, these antibodies might not be neutralizing antibodies.

Keywords: Anti-EPO antibodies, Chronic Renal Failure, Hypertension, Peshawar.

INTRODUCTION

Chronic Kidney Disease is a grave public health concern globally. Incidence rates are in Iceland 5%, in Norway 10%, in the United States 16.8%, 20 % in India, and 14 % in Pakistan (1). End-stage renal disease (ESRD) in Pakistan get elevate annually by approximation of 150 patients per million(2). People on hemodialysis (HD) are presumably to suffer from anemia than others with chronic renal failure (CRF). Having anemia of CRF poses a high risk of hospitalization and failure to thrive, as well as fatal cardiovascular complications. Anemia in patients with chronic kidney disease is multifactorial, but often due to a decrease in erythropoietin production by diseased kidneys (3). EPO promotes the formation of RBCs the bone marrow. Mean while, the mainstay of treatment for anemia of CRF is recombinant human EPO (rHuEPO). For many years, blood transfusions were the only treatment for renal anemia. These transfusions had a variety of complications and adverse effects, which led to the development of rHuEPO (4). After four weeks, hemoglobin and reticulocyte count are used to assess the response to recombinant EPO therapy. The optimal response to therapy is defined as an elevation in the level of hemoglobinof over 1.0 g/dl or an increase in absolute reticulocyte count of over 40*109/L (5)(6). Iron deficiency is one of the contributing factors to EPO resistance. Anemia of CKD is associated with a loss of 2-4 grams of iron per year among 25-38% of patients. This is because the underlying inflammatory process releases hepcidin in the liver, which prevents macrophages from releasing iron. By ascertaining the existence of iron-restricted erythropoiesis (7) and measuring the hemoglobin content of reticulocytes, an imbalance between EPO treatment and iron availability can be identified early. Increasing EPO's responsiveness requires iron therapy. Patients with a level of serum ferritin less than 100 ng/ml are considered to be iron deficient (8). Dialysis patients with CKD and low TIBC have poor clinical outcomes. Anti-EPO antibodies formed against rHuEPO therapy are the main cause for resistance. They can cause harsh anemia, diminishing hemoglobin levels and circulating reticulocytes by reacting with endogenous EPO. Pure red cell aplasia, where there is no production of RCs, can also result from its persistence in the body(9). Immunological tests, such as ELIZA and RIA, can detect anti-EPO antibodies in clinical labs very easily today. According to an Indian study, 69% of individuals with certain Renal problems had detectable anti-EPO antibodies(10).

The purpose of the present study was to make known the prevalence of anti-EPO antibodies in patients on dialysis and to assess if anti-EPO antibodies correlate with

haemoglobin (Hb) levels and other laboratory parameters. Clinicians and nephrologists may use the results to conceive a plan for discontinuing treatment with EPO with probable EPO antibody testing as a normal workup for patients in these situations.

METHODS AND MATERIALS

At the Department of Pathology, in Khyber Teaching Hospital, Peshawar, Pakistan, this cross-sectional study was performed from January, 2021 to December, 2021. We selected 150 patients through convenient sampling from the hemodialysis of Khyber Teaching Hospital, Peshawar. We obtained written informed consent from CRF patients who were 20 years of age or more, either male or female, going through haemodialysis while using recombinant EPO remedy for over semiyearly. Infection, inflammation, bleeding, hemolytic anemia, and hematological malignancies were excluded from the study.

An aseptic method was used to collect 05ml of venous blood by phlebotomists. Approximately 2 ml of blood was dispensed into an EDTA vacutainer for CBC and reticulocyte counts, and 3 ml for ELISA tests including Serum Ferritin, Iron, and Total Iron Binding Capacity. We examined FBC, Reticulocyte's count, Anti-Erythropoietin Antibodies, Serum Ferritin and Iron, T-SAT, and TIBC for this study. SPSS 25.0 statistical tool was used to interpret the data. We grouped Hb (Hemoglobin), Serum Iron, Ferritin, and TIBC as a percentage and frequency according to the HTN, diabetes mellitus, transfusion history, and HD rate and time span. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Out of total 150 patients, 79 (52.7%) were males and 71 (47.3%) were females. Moreover, age wise we categorized all the participants into three groups; group first range from 21-40 years, group second 41-60 years while group third ranges from 61-80 years with frequencies of 24 (16%), 93 (62%), 33 (22%) respectively (Table. 01). There were a variety of underlying conditions in our study including hypertension 65(43.3%), diabetes mellitus 32(21.3%), both hypertension and diabetes mellitus 10(6.7%), and gallstones disease called cholelithiasis, Congestive Heart failure, severe glomerulonephritis, and pyrexia 43(28.7%) (Table. 01).

Table 1: Demographic details of the participants

Variable	Parameters	Value	Percentages
Gender	Male	79	52.7%
	Female	71	47.3%
Age Group	21-40 years	24	16%
	41-60 years	93	62%
	61-80 years	33	22%
Co-morbidities	Hypertension	78	52%
	Diabetes	8	5.3%
	Hypertension and Diabetes	52	34.7%
	None	12	8%
Etiology	Hypertension	65	43.3%
	Diabetes	32	21.3%
	Hypertension and Diabetes	10	6.7%
	MiscellaneousGroup	43	28.7%

We divided our patients into three groups based on their dialysis duration: group1 (fifths of five years), group2 (fifths of five and above) and group3 (10 years and over), with 72(48%), 46(30.6%) and 32(21.3%) patients, respectively (Table. 02). There were 56 (37.3%) patients who received dialysis twice a week, while the rest 94(62.6%) received dialysis thrice a week (Table. 02). The transfusion history was found positive for 136(90.7%) patients while the remaining 14(9.3%) did not get transfused (Table. 02). Figure 1 shows that Anti-Erythropoietin antibodies were found in 92 (61.3%) individuals, while the antibodies were missing in 48 individuals (38.6%). There was no significant association found between gender and anti-EPO antibodies (Table. 03).

Table 2: Dialysis and transfusion history of the patients

Variables	Parameters	Values	Percentages
Transfusion History	Yes	136	90.7%
	No	14	9.3%
Dialysis Duration	<5 Years	72	48%
	5 Years and Above	46	30.6%
	10 Years and Above	32	21.3%
Treatment Duration	<5 Years	83	55.3%
	5 Years and Above	44	21.3%
	10 Years and Above	23	15.33%
Dialysis Frequency	Two times a week	56	37.3%
	Three times a week	94	62.6%
Frequency of Anti-EPO Antibodies	Yes	92	61.3%
	No	58	38.7%

Table 3: Association between Anti-EPO Antibodies and Gender

	With Anti-EPO Antibodies	Without Anti-EPO Antibodies	P-value
Males	49 (56.97%)	37 (43.02%)	0.2041
Females	43(67.2%)	21(32.81%)	
Total	92(61.3%)	58(38.6%)	Total 150 (100%)

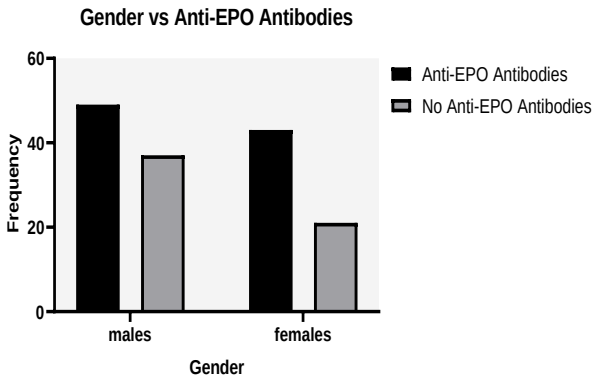

Figure 1: Association between Anti-EPO antibodies and Gender

DISCUSSION

In our study, we gave recombinant erythropoietin to patients with CKD on dialysis to shun blood transfusion, which could cause infection as well as iron overload. Nevertheless, the most of studies concerning the curing of low Hemoglobin (Anemia) with Erythropoietin in individuals on dialysis have revealed that such patients develop anti-

EPO antibodies. Symptoms of severe anemia can be caused by these antibodies reacting with the patient's own EPO(10). V Puri, in 2004, revealed that among 75 renal failure patients being treated with rHuEPO, fifty two were positive for anti-erythropoietin antibodies (11). Our results showing 92/150 patients having anti-EPO antibodies are similar to the results of that study. According to El Din et al, amongstninety patients with renal failure, 35 were found to have Anti-Erythropoietin antibodies (12). Zaki et al confirm the causes of low Hemoglobin in individuals with kidneys failure are multifaceted moreover erythropoietin manufacturing solely may not be sufficient to produce sufficient red blood cells (RBCs), which is a crucial reason causing anemia. The present study found that the gender difference between the anti-erythropoietin positive and anti EPO negative groups was insignificant statistically (p-value of 0.45), that is consistent with preceding studies. In addition, no significance difference was detected between the subsequent two age groups. These results are consistent with those of the El din and Alqahwaji studies. Possibly the outcomes are imitated by the small sample size (11, 13). In our study, hematological parameters such as hemoglobin were low in both groups, indicating that whole individuals were anemic, however the individuals with anti-EPO and those without the antibodies were found with no significant difference (p =0.84) between patients with anti-EPO antibodies and those without. This indicator of the production of RBCs in the bone marrow was stunted in patients with Anti-Erythropoietin Antibodies as compared to those with anti-EPO antibodies but this contrast was not statistically important. No crucial change in reticulocyte count between renal failure individuals with positive and negative anti-EPO antibodies in a previous study(14). Compared to another previous study, the result of these hematological parameters in our study was similar. In the previous study, Hb and reticulocyte counts were low in patients with anti-EPO antibodies. Low reticulocyte count in anti-EPO antibodies positive group suggests anemia mediated by EPO antibodies. Currently, anti-EPO antibodies do not significantly correlate with Hb and reticulocytes. Iranian research supports this finding. The researchers observed no correlation between anti-EPO antibodies and Hb levels (14). Kulkarni observed an inverse correlation between anti-EPO antibodies and hemoglobin levels in a study. As a result, we can attribute the slight reduction in hematological parameters (Hb and Retic) observed in our study to geographical differences, ethnicity, lifestyles and environmental factors(15). Our study showed no significant correlation between serum Urea and Creatinine levels and anti EPO antibodies, concurring with previous studies that showed same outcomes.

Anti-EPO antibodies positive individuals had median serum ferritin levels of 120(279-40) and anti-EPO antibodies negative individuals had median serum ferritin levels of 154.4(250-59.4), with no significance statistically (p-value=0.87). This is in line with the findings of the prior study.(16). It is important to investigate this issue further regarding iron stores. The results of a study conducted by El Din and Alqahwaji were alike(13). There was a statistically significant difference (p-value=0.10) between the mean levels with IQR of TIBC in the presence of anti-EPO antibodies and the mean levels with IQR in the absence of anti-EPO antibodies. To agree with the study done by Zaki et, al, no significant dissimilarity was fount between the levels of TIBC and TSAT% in patients with and without antiEPO antibodies (5). TIBC was significantly correlated with anti-EPO antibodies with a pvalue of 0.01 in agreement with previous work by Alqahwaji(13). Statistically insignificant association was found between the hemodialysis duration and individuals with and without anti-EPO antibodies in this study. According to the results of the present study, the duration of hemodialysis in both anti-EPO antibodies positive and negative patients is not correlated with anti-EPO antibodies positivity or negativity (7). It is also considered more immunogenic to administer subcutaneously. We administered the same type of rHuEPO therapy to all patients, i.e. alpha type, by subcutaneous injection(18). Antibodies of both types affect immune systems differently. In order to neutralize the biological effects of therapeutic proteins, neutralizing antibodies bind to them and directly damage them. As a result, the bio-effects of these proteins are lowered. In contrast, non-neutralizing antibodies bind to specific antigenic sites that do not interfere with the therapeutic action of these medications. In our study, Anti Erythropoietin antibodies might not be neutralizing since despite their formation, these antibodies did not have a clinical effect on patients. In the four months following the detection of anti-EPO antibodies, we again observed a slight reduction in Hb value in all the patients with anti-EPO antibodies. After four months of follow up, the mean Hb was 9.9 g/dl rather than 10.2 g/dl at baseline. Following up on the follow up mean of Hb values after four months, a statistically insignificant difference (p=0.70) was observed.

CONCLUSIONS

Anti-erythropoietin antibodies may not be neutralizing since they did not have clinical consequences in our study despite their formation. Four months after the detection of anti-EPO antibodies, all the patients with anti-EPO antibodies again experienced a slight decrease in Hb value. At baseline, Hb was 10.2 g/dl, but after four months of follow up, it was 9.9 g/dl. A statistically insignificant difference was observed in the follow up mean of Hb values after four months.

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