Anti-erythropoietin antibodies among Sudanese Hemodialzed patients treated with recombinant human-erythropoietin

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Abstract

Erythropoietin is generally well tolerated in rare cases patients have developed anti-EPO antibodies that can be negatively impact safety and efficacy. Therefore the detection of antibodies against EPO is a regulatory requirement during clinical development and post approval. This was a cross sectional hospital based study to detect the anti-EPO antibodies among hemodialyzed patients in Sudan conducted during March - July 2017. A total of 88 serum samples were obtained from hemodialyzed patients referred from different areas all over Khartoum state in Hemodialyzed Unit, Iben-Sina Hospital. Patient's information was recorded in questionnaire before sampling. A volume of 3 ml venous blood was collected from each participant and anti-EPO antibodies were assessed by Enzyme Linked Immunosorbent Assay (ELISA) on serum samples. Over all seropositivity (more than normal range 0.781-50ng/ml) of anti-EPO antibodies in hemodialyzed patients was found to be 9.1%(8/88) with the same ratio of seropostivity between male and female (1:1).There were a correlation of borderline significance between the duration of hemodialysis (P value =0.05)and the duration of injection of recombinant EPO (P=0.04) versus the result of Anti-EPO antibodies among the patients. Based on the findings obtained from this preliminary study Anti-EPO antibody is existence in hemodialyzed Sudanese patients. Importantly, regular surveillance through screening to investigate hemodialyzed patients for anti-EPO antibodies is recommended.

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Introduction

Chronic kidney disease (CKD) is considered a public health problem worldwide with high incidence and prevalence rates. In end stage renal disease (ESRD), renal function must be replaced by dialysis or renal transplantation (Alves*etal*., 2015). Anaemia is a common complication of chronic kidney disease. The anaemia of kidney disease tends to develop once the glomerular filtration for creatinine clearance falls below 60 ml /min. Anaemic patients with a lesser degree of renal impairment should be screened carefully for another cause of anemia. The in-appropriately low circulation concentration of erythropoietin (EPO), a hormone largely produced by the peritubular cells of kidney, is the most important factor for anaemia (Macodougall, 2015). Human EPO is an acidicglycoprotein hormone with a molecular mass of 30.4 KDa consisting of 165 amino acids and four carbohydrate side (Jelkmann,2013). Synthetic EPO therapy has transformed the management of renal anemia; it induces an increase in haemoglobin concentration and prevents the need for repeated blood transfusion particularly in dialysis patients (Macodougall, 2015). EPO is generally well tolerated in rare cases patients have developed anti-EPO antibodies that can be negatively impact safety and efficacy. Human EPO induces neutralizing antibodies which not only cross-react with all EPO products but also neutralize the endogenously produced protein causing antibody-mediated pure red cell aplasia (PRCA) in chronic kidney disease patients (Casadevall *et al*, 2002). Antibodies of high- and low-affinity with binding specificity to both neutralizing and non-neutralizing EPO epitopes essentially represents non-neutralizing antibodies, usually pre-existingearly onset antibodies, typically non-neutralizing, IgM and IgG1, and those characteristic of a neutralizing antibody-mediated PRCA - IgG1, IgG2 and IgG4 isotypes (Mytych *et al.*, 2012). It should be noted however that the IgM antibodies generated by grafting of the CDR region of the IgG antibodies onto an IgM framework may not truly reflect the affinity/activity of IgM antibodies that arise in vivo (Wadhaw *etal.* 2015). It has been documented that the majority of patients treated with EPO to correct anemia of chronic renal failure developed anti-EPO antibodies, the detection of the antibodies to erythropoietin might help to have alternative way for therapy. The aim of the present study was to detect the incidence of anti-EPO antibodies in Sudanese patients on regular hemodialysis and injected EPO therapy.

Methods

This was a across- sectional hospital based study conducted on hemodialyzed section at the renal unit in Iben Siena Hospital, Khartoum, Sudan during March - July 2017. The study received ethical approval fromthe Renal Unit in Iben Siena hospital. From consented 88 patients on regular hemodialysis who come from different area of Khartoum state and injected recombinant human erythropoietin twice a weekly with different age and gender. A volume of 3 ml peripheral blood was collected into plain tubes and centrifuged for 20 minutes at 1000xg and sera were stored at -20 until analysis.Serum was tested for human anti-EPO antibody by Enzyme-linked immunosorbent assay (ELISA) kit according to manufacturers'instructions (Fine Biological Technology Co., Ltd, Wuhan). Briefly, EPOwas pre-coated onto 96 well plates and the Biotin-labeled EPO was used as detection antigen.Thestandards,test samples and Biotin labeled EPO were added to the wells subsequently,and wash with wash buffer.HRP-Streptavidin Conjugate was added and unbound conjugate were washed away with wash buffer TMB substrates were used visualize HRP enzymatic reaction.TMB was catalyzed by HRP to produce a blue colure product that changed into yellow after adding acidic stop solution.The density of yellow is proportional to the anti-EPO antibody amount of samples captured in plate. O.D. absorbance was read at 450 nm in a micro-platereader,and then the concentration of anti –EPO antibody calculated.as the range of antibody (0.781-50ng/ml).The patients were divided into three group according to the result H (above the range)9.1%, N (within the range) 52.3%l (below the range) 38.6%.

Statistical analysis

Statistical analysis of the data was performed using SPSS. Variables descriptively expressed as number and percent. The significance level at P value 0.05 using chi-square test .

Results

Characteristics of the study group

A total of 88 patients were randomly selected in the study with the majority (67%) were males. The patients'age ranged between 20-80 and most of the patients were on regular hemodialysis (71.6%) and injected human recombinant erythropoietin (69.3%) for 1-5 years (Table 1).

Table 1: Characteristics of the study group in relation to duration of dialysis and duration injection of rHuEPO

Variable	Frequency	Percent %
Gender:		
Male	59	67.0
Female	29	33.0
Age groups:		
Less than 20	2	2.3
20-40	27	30.7
41-60	48	54.5
61-80	11	12.5
Duration of Dialysis:-		
Less than 1 year	3	3.4
1-5 years	63	71.6
6-10 years	22	25.0
Duration of injection of rHuEPO:-		
Less than 1 year	12	13.6
1-5 years	61	69.3
6-10 years	15	17.0

Over half (52.3%) of the patients were within the normal range of anti-erythropoietin antibodies (0.781-50ng/ml) and 38.6 of the patients were within the lower range of antibodies compared to 9.1% above the higher range as shown in table 2 .

Table 2: (The Frequency and percent of anti-EPO antibodies result)

Sero-positivity of anti-erythropoietin antibodies:	Frequency	Percent%
H	8	9.1
N	46	52.3
L	34	38.6

Table 3: The rate of anti-EPO positivityaccording to the duration of hemodialysis and the Duration of injection

Durationof injection OfHuEPO	Range	Frequency	%
Less than 1 year	H	1	1.1
	N	8	9.1
	L	3	3.4
Total		12	13.6
1-5 years	H	3	3.4
	N	30	34.1
	L	28	31.8
Total		61	69.3
6-10 years	H	4	4.5
	N	8	9.1
	L	3	3.4
Total		15	17
Duration of Dialysis	Range	Frequency	%
Less than 1 year	H	0	0
	N	1	1.1
	L	2	2.3
Total		3	3.4
1-5 year	H	4	4.5
	N	30	34.1
	L	29	33
Total		63	71.6
6-10 year	H	4	4.5
	N	15	17
	L	3	3.4
Total		22	25

As shown in Table 3 the incidence of anti-erythropoietin antibodies were increased gradually according to the number of years of injection of the recombinant human erythropoietin.

A border line significant difference between the duration of hemodialysis (P=0.05) and the duration of injection of recombinant EPO (P=0.04) and the result of Anti-EPO antibodies in the recruited patients were found.

Discussion

Erythropoietin (EPO) is an endogenous growth factor that is absolutely required for erythropoiesis.Recombinant EPO has been successfully and safety used for almost 20 year to treat anemia in patients with end stage renal diseases (ESRD),chronic renal failure (CRF)and chemotherapy induced anemia in malignancy .Recently,antibody mediated red cell aplasia (PRCA) associated with administration of rHuEPO has been identified as a cause of a major resistance to erythropoietin (Puri, 2004).Our result showed that 8 patients (9.1%) had the anti-EPO antibodies in their blood above the range(Range is 0.781-50ng) while 46 patients(52.3%) within the range and 34patients(38.6%)under the range. These findings is in consistent with Puri study (2004) in India who found only 8% of the patients had the anti-EPO antibodies in their blood greater than 10%. Prior findings from other studies conducted by Castelli and colleagues (2000) who revealed 67%, EL-Din etal (2010)revealed an occurrence of 38.9% ,Alqahwajietal., (2014) revealed 22.5% occurrence of anti-EPO antibodies among patients receiving recombinant EPO for various renal complications.

The findings of lower percentage of anti-EPO anti-bodies positive patients in the present study compared to others may be explained by the relatively lower doses of EPO (2000 IU/ week) used to treat the patients relative to the doses used in the other studies,or may be the patient did not injected rHuEPO regularly also commercial preparation of EPO could affect the immune system differently as a different populations and ethnicities have different immune system (Watsonetal., 2013). Although of borderline significant, the present study found a relationship between the duration of hemodiaylsis (p=0,04) and the duration of rHuEPOinjection (P=0.05) with the positivetyof the anti-EPO antibodies in the patients.This is disagree with Alqahwaji et al.(2014) where there was not statically significant difference between the duration of hemodiaylsis and

result of anti-EPO antibodies of patients.Also this study showed that there is a statically significant difference between injected rHuEPO and result of anti-EPO antibodies of patients which in agreement with Alqahwajietal.(2014) who found a statically significant difference between the injected rHuEPO and result of anti-EPO antibodies of patients .

Conclusion

In conclusion, patients develops anti-EPO antibodies associated with the duration of hemodialyzed and with duration of EPO injection with high frequency among the males.

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