International Journal of Medicine Research
ISSN: 2455-7404
www.medicinesjournal.com
Volume 3; Issue 1; January 2018; Page No. 06-09

Erythropoietin effects on anaemia status and hepcidin levels in chronic kidney disease

Herviani Sari[1], Harun Rasyid Lubis[2], Lukman Hakim Zain[3], Adi Koesoema Aman[4], Dr. Stephen Chee Liang Koh[5*], Herman Hariman[6]

[1] Department of Bio Medics, School of Medicine, University of North Sumatera, Medan, Indonesia
[2] Division of Nephrology, Department of Internal Medicine, School of Medicine, University of North Sumatera, Medan, Indonesia
[3] Division of Hepato-gastroenterology, Department of Internal Medicine, School of Medicine, University of North Sumatera, Medan, Indonesia
[4, 5, 6] Division of Haematology, Department of Clinical Pathology, School of Medicine, University of North Sumatera, Medan, Indonesia

Abstract
Background: Anaemia occurs early in kidney disease which worsens with declining kidney function. Erythropoietin (EPO) is now widely used to correct anaemia including chronic kidney disease. Inflammation and infection increase hepcidin synthesis. The aim of the study is to determine the first dose effect of erythropoietin administration on anaemia status and hepcidin levels in chronic kidney disease patients undergoing haemodialysis.
Materials and Methods: 25 patients were recruited (males n= 19, females n=6). Their mean age was 51.9 ± 8.0 years ranging between 33 and 63 years. Blood sampling was performed after three months of haemodialysis (pre-EPO) immediately followed by EPO injection and blood sampling 3 days thereafter. Haemoglobin, reticulocyte and hepcidin levels were including Pearson's correlation were determined.
Results and Discussion: Significant mild improvement in haemoglobin levels from mean 8.1 g/dL to 8.5 g/dL (*P*=<0.05) was seen and improved levels seen in only 76% of patients. Reticulocytes showed a mean rise from 1.02% to mean 1.33% *(P*=<0.01) and improved levels was seen in 80% of patients. Hepcidin levels showed a reduced trend from mean 72.5 ng. mL to 51.4 ng/mL but did not reach statistical significance *(P*=0.15). No change in hepcidin levels was seen in 64% of patients whilst reduced levels in 20% and further rise in 16% of patients. No significant correlation between the parameters studied was seen.
Conclusion: The first EPO administration showed mild improved benefits in anaemia status but hepcidin levels improved in only 20% patients which need to be further determined after more EPO administration.

Keywords: EPO, haemoglobin, reticulocytes and hepcidin in chronic kidney disease

Introduction

Chronic kidney disease is considered a public health problem worldwide with high incidence and prevalence of kidney failures with poor outcomes. In chronic kidney disease Stage 5, renal function must be replaced by dialysis or kidney transplantation. Anaemia of chronic kidney disease (CKD) in most patients were mainly of normocytic normochromic red blood cells and principally due to reduced renal erythropoietin (EPO) production by EPO producing kidney cells and to a lesser extent to shortened red blood cell survival and iron deficiency [1]. Currently, data regarding effective management of anaemia in CKD patients on dialysis is inadequate [1]. Erythropoietin is recommended in individuals progressing towards Stage 5 renal disease in correcting anaemia and its complications commonly seen in this disease [2]. EPO is a hormone produced by the kidney, it promotes formation of red blood cells in the bone marrow to initiate synthesis of haemoglobin. Ninety-percent of erythropoietin is produced by the kidney renal cortex and hypoxia increase EPO production and subsequently erythropoiesis [3, 4] EPO is now widely used to treat anaemia associated with a range of conditions including CKD [5]. It can be synthesized and used as treatment of some forms of anaemia but has also been misused as an enhancing performance drug by some athletes in competition. Systemic inflammation leads to production of inflammatory mediators, interleukins and tumour necrotic factor (TNF) which affects the proper function of erythropoietin in the bone marrow [6]. Careful evaluation of the subject's iron stores is necessary to utilize erythropoiesis effectively. Reticulocytes, the product of erythropoiesis in the bone marrow enters the circulation within 24 hours, the reticulum is expelled followed by significant reduction in reticulocyte count. Following EPO administration reticulocyte numbers begin to increase after a dose-independent delay of around 1.7 days have been reported [7]. Hepcidin, a peptide hormone is synthesized mainly in the liver and a key regulatory of iron entry into the circulation in mammals [8]. It blocks intestinal absorption of iron and the release from iron stores by inducing the internalization and degradation of the cellular iron exporter ferroportin [9]. Inflammation and infection increase hepcidin synthesis. Kidney function play an important role in hepcidin clearance and kidney dysfunction results in decreased clearance [10]. Hepcidin better known for its role in iron homeostasis is increased by iron loading, inflammation, hypoxia and

erythropoiesis [11, 12] whereas iron deficiency and blood loss, reduce hepcidin expression [13, 14]. EPO administration suppresses circulating hepcidin levels within two days [15] and the suppression is thought to be mediated by increase bone marrow activity [16]. The extent of hepcidin suppression showed that EPO administration to be a useful therapy that is currently accepted for anaemia of chronic disease [17]. Hepcidin has been suggested as being a biomarker of iron status and iron demand in dialysis patients and the levels may have a predictive value in individual CKD patients [15, 18-20].

The aim of the study is to determine the first dose effect of erythropoietin administration on anaemia status and hepcidin levels in chronic kidney disease patients undergoing haemodialysis.

Materials and Methods

The study received ethical approval from the Health Research Ethical Committee (159/KOMET/FKUSU/2015), Faculty of Medicine, University of North Sumatera, Indonesia. It was conducted at the Department of Clinical Pathology, Faculty of Medicine, University of North Sumatera/Haj Adam Malik Hospital.

Inclusion criteria: The Indonesian Government National Health Insurance allows only haemodialysis patients to receive free erythropoietin injection only after they had undergone three months of haemodialysis, greater than 18 years old, haemoglobin <11.0 g/dL but not in a state of iron deficiency, serum Ferritin >100µg/L, transferrin saturation >20%, patients should have Grade V KIDGO and e GFR <15ml/min/1.73m^2. Exclusion criteria: if any of the above inclusion criteria are not met.

Subjects. 25 patients having met the above Inclusion Criteria were recruited (males n= 19, females n=6) after having given written Informed Consent. Their mean age was 51.9 ± 8.0 years ranging between 33 and 63 years. The first-time administration of recombinant human Erythropoietin (EPREX, Jason-Cilag) 2,000 units were given by subcutaneous injection to the patients immediately after three months of haemodialysis and at every haemodialysis cycle thereafter. Iron was not administered to the study patients as all patients had normal serum iron levels.

Blood sampling. About 10 mL of blood from a clean venipuncture was obtained and drawn into 2.5 mL EDTA vacutainer tubes for haemoglobin estimation and reticulocyte count and the remainder into a plain tube. Blood was left to clot for about an hour at room temperature, then centrifuged at 2000g for 30 minutes. The serum was aliquoted and stored at -70^{o}C for further analysis. Blood sampling was performed after three months of haemodialysis (pre-EPO) and 3 days following haemodialysis after first EPO administration (post-EPO). Laboratory analysis. Haemoglobin and reticulocyte was determined by the automated Haematology Analyzer Sysmex XN1000 (version XN10 and XN20). Enzyme-linked immunoassay (ELISA) was used to determine the levels of Hepcidin (Elabscience Biotechnology, Bethesda, USA).

Statistical analysis. The Statistical Package for Social Sciences (SPSS 22 IBM Corp) was used to perform statistical analysis. The group mean paired samples t-test and Mann-Whitney test for differences between groups and Pearson correlation was also performed. A P-value of <0.05 was considered statistically significant.

Results

Characteristics of patients on haemodialysis receiving first time erythropoietin

Twenty-five patients (male n= 19, female n=6) who fulfilled the inclusion criteria and gave written Informed Consent were recruited to receive the first-time erythropoietin administration after having gone through three months of haemodialysis as required in the inclusion criteria. Their mean age was 51.9 ± 8.0 years and ranged between 33 and 63 years. The underlying disease and clinical symptoms are shown in Table 1.

Haemoglobin, reticulocytes and hepcidin levels in patients who had undergone three months of haemodialysis (pre-EPO) and three days after receiving the first-time administration of erythropoietin (post-EPO) were compared.

Statistical significant increase in haemoglobin levels (P=<0.05) from mean 8.1g/dL to mean 8.5 g/dL (4.9% increase) post-EPO administration was seen. However, the level is still considered severely anaemic. Reticulocytes, however, showed a more significant improvement rising from a mean of 1.02% to mean 1.33% (30.4% increase, *P*=<0.01)) after EPO administration. Hepcidin levels although showed a mean decrease from 72.5 ng/mL to mean 51.4 ng/mL (29.1% reduction, P=0.15), it did not reach statistical significance probably due to the wide variation in results seen. The mean level is still above the normal range of <47 ng/mL (Table 2).

Evaluation on the effects of first dose of erythropoietin on the levels of haemoglobin, reticulocytes and hepcidin (Table 3).

Haemoglobin. Only nineteen (76%) of patients showed response of increased haemoglobin levels following EPO administration and 6 (24%) patients had reduced levels.

Reticulocytes. Twenty (80%) of patients responded to increase reticulocytes whilst five (20%) had reduced levels following EPO administration.

Hepcidin. Sixteen (64%) of patients showed no change in hepcidin levels whilst five (20%) showed reduced levels and four (16%) had elevated levels following EPO administration. No change in hepcidin levels was observed in the other sixteen (64%) patients.

Correlation studies. Pearson's correlation was done and no significant correlation were seen between haemoglobin and reticulocytes (r= -0.217, *P*= 0.13), haemoglobin and hepcidin (r=0.169, *P*=0.24), hepcidin and reticulocytes (r= -0.066, *P*=0.65)

Table 1: Characteristics of patients on haemodialysis receiving first time erythropoietin

N 25
Sex: male n = 19, female n = 6
Age mean ± SD: 51.9 ± 8.0 years, range: 33 - 63 years
Underlying disease:
Hypertension nephropathy n = 11

Diabetic nephropathy n = 10
Chronic glomerulonephritis n = 2
Chronic pyelonephritis n = 1
Obstructive infective kidney disease n = 1
Clinical symptoms:
Dizziness/weakness/fatigue/nausea n= 23
Dizziness/ weakness/reduced appetite n = 2

Table 2: Haemoglobin, reticulocytes and hepcidin levels in patients undergone 3 months of haemodialysis (pre-EPO) and 3 days after receiving the first administration of erythropoietin (post-EPO) were compared.

	Pre-EPO	**Post-EPO**	**P**
N	25	25	
Haemoglobin			
Mean (SD) g/dL	8.1 (1.0)	8.5 (1.2)	<0.05
Range g/dL	6.2 – 10.3	6.4 – 10.9	
Reticulocytes			
Mean (SD) %	1.02 (0.60)	1.33 (0.67)	<0.05
Range %	0.31 – 1.98	0.48 – 2.65	
Reticulocytes			
Mean (SD) %	72.5 (76.0)	51.4 (32.3)	0.15
Range %	17.79 -337.81	2.85 – 123.45	

Table 3: Evaluation on the effects of first dose of erythropoietin administration on the levels of haemoglobin, reticulocytes and hepcidin.

	Haemoglobin	**Reticulocytes**	**Hepcidin N=25**
Reduced from pre-EPO n	6 (24%)	5 (20%)	5 (20%)
Elevated from pre-EPO n	19 (76%)	20 (80%)	4 (16%)
No change from pre EPO n	-	-	16 (64%)

Discussion

Chronic kidney disease is considered a public health problem worldwide with high incidence and prevalence of kidney failures with poor outcomes. In chronic kidney disease Stage 5, renal function must be replaced by dialysis or kidney transplantation, however, life expectancy is shorter than that of the general population with similar demographics [20, 21]. Anaemia occurs early in the development of kidney disease which worsens with declining kidney function. EPO administration is essential for erythropoiesis and recommended in individuals progressing towards Stage 5 renal disease to correct anaemia and its complications. It serves as stimulants for growth of specific types of blood cells in the bone marrow to produce more red blood cells and initiate synthesis of haemoglobin. EPO is now widely used to correct anaemia associated with a range of conditions including CKD [5]. Following EPO administration reticulocyte numbers begin to increase after a dose-independent delay of around 1.7 days have been reported [7]. In our study, following three days after EPO administration haemoglobin levels showed statistically significant mild improvement in only 76% (19/25) of patients and reticulocytes improvement in 80% (20/25) patients. Moreover, severe anaemia persist in these patients. EPO administration suppresses circulating hepcidin levels within two days have been reported [15]. In our study hepcidin levels after first EPO administration showed a mean 29.1% reduction decreasing from mean 72.5 ng/mL to mean 51.4 ng/mL but they did not reach statistical significance. Upon further evaluation, 64% (16/15) of patients showed no change in hepcidin levels whilst 20% (5/2) patients showed reduced levels and 16% (4/25) patients had further elevated levels. No significant correlation was seen between haemoglobin with reticulocytes and hepcidin and between hepcidin and reticulocytes. The suppression of hepcidin level is probably mediated by increase bone marrow activity have been suggested [16]. The extent of hepcidin suppression showed that EPO administration to be a useful therapy that is currently accepted for anaemia of CKD [17] but is not strongly supported in our study. The study showed that further EPO administration will improve the anaemia status and the reduction in hepcidin levels needs to be determined. However, subsequent study for continued EPO administration was not carried out.

Conclusion

The first EPO administration showed benefits in CKD patients undergoing haemodialysis in bone marrow erythropoiesis and improving anaemia status whilst further reduction on high hepcidin levels needs to be determined.

Acknowledgments

We wish to express our sincere gratitude to Mrs Junita Siahaan for her expert technical assistance

Conflict of Interest

The authors declared that they have no Conflict of Interest.

References

1. Srinivasan R, Fredy IC, Chandrashekan S, *et al*. Assessment of erythropoietin treatment of anemia in chronic kidney failure-ESRD patients. Biomed Pharmacother. 2016; 82:44-8.
2. Esbach JW, Haley HR, Adamson JW. New insights into the treatment of the anemia of chronic renal failure with erythropoietin. Semin Dial. 1990; 3:112-21.
3. Peyssonnaux C, Zinkernagel AS, Scheupbach RA, *et al*. Regulation of iron homeostasis by hypoxia-inducible transcription factor (HIFs). J Clin Invest. 2007; 117:1926-32.
4. Jelkman W. Regulation of erythropoietin production. The Journal of Physiology, 2011doi:10.1113/jphysiol, 2010 19505.
5. Hodges VM, Rainey S, Lappin TR, Maxwell AP. Pathophysiology of anaemia and erythrocytosis. Crit Rev Oncol/Hematol. 2007; 64:139-58.
6. Al-Khoury S, Afzali B, Shah N, *et al*. Diabetes, kidney disease and anemia; time to tackle a troublesome triad? Int J Clin Path. 2007; 61:281-9.

7. Krzyzanski W, Perez-Ruixo JJ. An assessment of recombinant human erythropoietin effect on reticulocyte production rate and lifespan distribution in healthy individuals. Pharm Res. 2007; 24:758-72.
8. Ganz T. Hepcidin, a key regulator of iron metabolism and mediator of anemia of inflammation. Blood. 2003; 102:783-8.
9. Nemeth E, Tuttle MS, Powelson J, *et al*. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalisation. Science. 2004; 306:2090-3.
10. Zaritsky J, Young B, Gale b, Wang HB, *et al*. Reduction of serum hepcidin by hemodialysis in paediatric and adult patients. Clin J Am Soc Nephrol. 2010; 5:1010-4.
11. Nemeth E and Ganz T. The role of hepcidin in iron metabolism. Acta Haematol. 2009; 122:78-80.
12. Zhoa N, Zheng AS, Enns CA. Iron regulation by hepcidin. J Clin Invest. 2013; 123:2337-43.
13. Darshan D, Anderson GJ. Interacting signals in the control of hepcidin expression. Biometals. 2009; 22:77-87.
14. Pak M, Lopez MA, Gabayan V, Ganz T, Rivera S. Suppression of hepcidin during anemia requires erythropoietin activity. Blood. 2006; 108:3730-5
15. Ashby DR, Gale DP, Busridge M *et al*. Plasma hepcidin level are elevated but responsive to erythropoietin therapy in renal disease. Kidney Int. 2009; 75:976-81.
16. Vokurka M, Krist J, Sulc K, Necas E. Hepcidin mRNA levels in mouse liver response to inhibition of erythropoiesis. Physiol Res. 2006; 55:667-74.
17. Ashby DR, Gale DP, Busridge M, *et al*. Erythropoietin administration in humans caused a marked and prolonged reduction in circulating hepcidin. Haematologia. 2013; 95:505-8.
18. Zaritsky J, Young B, Wang HJ, *et al*. Hepcidin, a potential novel biomarker for iron status in chronic kidney disease. Clin J Am Soc Nephrol. 2009; 4:1051-6.
19. Weiss G, Theuel I, Eden S, *et al*. Serum hepcidin concentration in chronic haemodialysis patients associations and effect of dialysis, iron and erythropoietin therapy. Eur J Clin Invest. 2009; 39:883-90.
20. Van der Putten K, Jiek E, van der Bloek D, *et al*. Hepcidin-25 is a marker of the response rather than resistance to exogenous erythropoietin in chronic kidney disease//chronic heart failure patients. Eur J Heart Fail. 2010; 12:943-50.
21. The National Institute of Health, Concensus of Conference Statement. Morbidity and mortality of renal dialysis. An NIH Concensus Conference Statement, Ann Intern Med. 1994; 121(1):62-70.
22. Pozzoni P, Del Cecchio L, Pontorieo G, *et al*. Long-term outcome in hemodialysis morbidity and mortality. J Nephrology. 2004; 17:S87-S95.