Original Article

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Internal Medicine Section

Role of Hepcidin on Response of Erythropoietin Stimulating Agents in Anaemic Advanced Chronic Kidney Disease Patients

SATYENDRA KUMAR SONKAR[1], ANIL KUMAR[2], NEERAJ KUMAR SINGH[3], GYANENDRA KUMAR SONKAR[4], SANT PANDEY[5], VIVEK BHOSALE[6]

ABSTRACT

Introduction: Anaemia contributes significantly to the morbidity and mortality in Chronic Kidney Diseases (CKD). Erythropoietin Stimulating Agents (ESA) have revolutionised the management of the anaemia in CKD patients. However, over 50% of the patients do not reach the target haemoglobin level. Hepcidin which plays important role in iron metabolism and inflammation may be the cause of this hyporesponsiveness to ESA.

Aim: The present study was aimed to assess possible relation between hepcidin and erythropoietin therapy with special attention to hyporesponsive anaemia in advanced kidney disease.

Materials and Methods: In this cross sectional study, total of 81 clinically stable patients from Department of Medicine and Nephrology, King George's Medical University, Lucknow of advanced CKD on ESA for past 12 weeks were enrolled. Patients whose haemoglobin rose more than 2 gm/dL or were above 10 gm/dL were classified as responders while below 10 gm/dL were classified as poor responders.

Results: In present study, majority 54 patients (66.7%) were poor responders to ESA. We found significant higher level of hepcidin in poor responders (p=0.01) while no significant variation in serum iron, Transferrin Saturation (TSAT) and ferritin. High sensitivity C-Reactive Protein (hsCRP) was markedly elevated with median value 3.13 mg/L and Interquartile Range (IQR) (1.14-13.78) in all patients.

Conclusion: Hepcidin may be one of the causes of hyporesponsiveness to ESA due to reticulo-endothelial blockade of iron.

Keywords: Anaemia, Haemodialysis, Inflammatory markers, Iron

INTRODUCTION

Anaemia contributes significantly to the morbidity and mortality in Chronic Kidney Diseases (CKD). The chief aetiology of anaemia in CKD is Erythropoietin (EPO) deficiency. Adequate iron stores are essential for achieving maximum benefits from the Erythropoietin-Stimulating Agents' (ESA) therapy. Despite the widespread EPO use, over 50% of the patients do not reach the target haemoglobin levels [1,2]. This hyporesponsiveness to EPO is associated with increased mortality [3]. Iron deficiency and inflammation are common causes for poor response to Epo therapy [4]. For assessment of degree of inflammation CRP which is an acute phase reactant can be used as an inflammatory marker [5]. Hepcidin has shown to be a regulator of body iron stores and hence may play an important role in pathogenesis of anemia in patients of chronic kidney disease [6].

Ferritin also acts as an acute phase reactant and hence get increased in acute inflammation, so it can not be reliably used as a marker of body iron stores [7]. To overcome the poor absorption and utilization of oral iron, parenteral iron therapy has shown to improve body iron stores and thus reduction of Epo requirement. However by administering parenteral iron there can be increased morbidity and mortality by induction of a pro-inflammatory state, due to increased oxidative stress [8,9]. On account of inflammatory activation the assessment of iron profile by ferritin may be dubious.

Hepcidin is the main hormone responsible for maintaining systemic iron homeostasis. Produced by the liver and secreted into circulation, hepcidin binds and induces degradation of the iron exporter, ferroportin, on duodenal enterocytes, reticuloendothelial macrophages, and hepatocytes to inhibit iron entry into the plasma. Inflammatory cytokines directly induce hepcidin transcription, presumably as a mechanism to sequester iron from invading pathogens, leading to the iron sequestration, hypoferremia, and anaemia that are hallmarks of many chronic diseases including CKD [10].

The treatment of anaemia in CKD patients usually involves the use of recombinant human erythropoietin (rHuEPO). The main cause of rHuEPO treatment failure is the low iron availability [11]. Despite rHuEPO and intravenous iron in the majority of patients, the prevalence of anaemia is high. This indicates the existence of other important factors related to rHuEPO resistance.

In this study, evaluation of the iron stores, degree of inflammatory activation and hepcidin levels in patients with advanced chronic kidney disease was done. TSAT, ferritin and hepcidin were looked as a marker for poor response to rHuEPO.

MATERIALS AND METHODS

The study was conducted in the Department of Medicine and Nephrology, King George's Medical University, Lucknow. It was a cross sectional study of one year duration from August 2016 to July 2017. Sample size of 81 patients was calculated from epitools online calculator. Patients >18 years and <65 years in stable clinical state in advanced CKD (stage 4 and 5), eGFR (estimated glomerular filtration rate) <30 mL/min calculated from equation from the Modification of Diet in Renal Disease (MDRD) were selected. Patients whose transferrin saturation (TSAT) was >20%, ferritin between 100-300 µg/L, haemoglobin ≥7 but <10 g/dl and who had taken in a maximum dose of 4000 IU weekly for at least 12 weeks were selected. Vitamin B12 and Folic acid was normal in all patients. Patient having active infection, active cancer,

thrombosis or inflammation, blood transfusions within three months, immunosuppressive therapy, acute cardiovascular complication (including acute coronary syndrome, acute heart failure) were excluded. Patients whose haemoglobin rose more than 2 gm/dL or were above 10 gm/dL were classified as responders while below 10 gm/dL were classified as poor responders.

All venous blood samples (10 mL) were taken by laboratory technician in the morning after an overnight fast. Haematological measurements were made in 7 mL of fresh venous blood with EDTA and clotted blood. Haemoglobin concentration (Hb), Red Blood Count (RBC), Haematocrit (HCT), platelets count, iron profile, lipid profile, creatinine, urea and uric acid were measured using standard laboratory methods (automated system) in a central laboratory. Remaining 3 mL of the plasma and serum was centrifuged and frozen at –70°C until further laboratory analysis. Commercially available kits were used to measure hepcidin-25 and hsCRP (by sandwich ELISA method of Sunred, Shanghai). Above study was approved by institutional ethical committee and written consent was taken from every patient.

STATISTICAL ANALYSIS

Baseline characteristics were assessed with standard descriptive statistics. eGFR was calculated from MDRD formula. Normality of data was tested by Kolmogorov–Smirnov test. If the normality was rejected then non-parametric test was used. Continuous variables were presented as mean±standard deviation and median with Interquartile Range (IQR) (as applicable). Quantitative variables were compared using independent t-test and Mann–Whitney test (for non-parametric data) between two groups. For Non-parametric data, log of data was used to find out the correlation. For quantifying association, odds ratio with 95% confidence interval was used and significance of odds ratio was calculated by Fisher's-Exact Probability statistic. The data were entered in MS EXCEL spread sheet, and analysis was done using advanced statistical tool of Microsoft Excel 2010. p<0.05 was considered statistically significant.

RESULTS

In our study 81 patients were enrolled. Mean age of the patients was 48.3±14.0 years. Male to female ratio was 2:1 i.e., (54 vs 27). Majority of patients (45%) were of diabetic kidney disease and rest were of chronic glomerulonephritis (25%), chronic interstitial nephritis (10%) and hypertensive nephropathy (5%) while in 15% of patient aetiology was not known. 52% (42) of the enrolled patients were on maintenance haemodialysis (MHD) for at least twice in a week and rest 48% (39) were not on dialysis.

[Table/Fig-1] summarizes various parameters between responders and poor responders in advanced CKD patient on rHuEPO. In [Table/Fig-1], 27 (33.3%) patients were responders and 54 (66.7%) patients were poor responders to rHuEPO (Epoetin alfa). Mean age in responder Vs poor responders was 51.66±8.8 years vs. 46.61±12.07 years. Among responders 66.7% (18) were male while in poor responders also 66.7% (36) were male (odds ratio 1.03, p=0.95). Out of total 36 diabetic patients 11 (40.7%) were among responders while 25 (46.3%) were among poor responders (odds ratio 1.25, p=0.63) [Table/Fig-2]. Age, gender and diabetes did not affect the response to EPO. However, patients with low eGFR/ raised creatinine were poor responders. Iron, TSAT had no significant difference while serum albumin and TIBC was significantly decreased in poor responders. Patients on MHD were poor responder (39) i.e., 72.2% when compared to non MHD (odds ratio 20.8, p<0.001) [Table/Fig-2]. There was no significant difference in hsCRP level between the groups but hepcidin was significantly higher in poor responder group than responder group which may suggest role of hepcidin in poor response to erythropoietin.

	Responders (n=27, 33.3%)	Poor responders (n=54, 66.7%)	p-value
	Mean±SD/ Median (IQR)	Mean±SD/ Median (IQR)	
Haemoglobin (g/dL)	11.50±1.85	8.24±0.93	**<0.001**
Age (years)	51.66±8.8	46.61±12.07	0.23
Gender (Male)	18 (66.7%)	36 (66.7%)	>0.05
Diabetic	11 (40.7%)	25 (46.3%)	0.63
S. Creatinine (mg/dL)	4.43±2.56	7.08±3.30	**0.03**
Log eGFR	1.18±0.31	0.94±0.27	**0.05**
S. Iron (µg/dL)	68.70±25.96	75.06±54.41	0.70
TIBC (µg/dL)	284.88±67.13	225.38±39.36	**0.05**
TSAT	22.48±6.72	21.46±4.00	0.72
S. Ferritin (µg/L)	110 (106-325)	208 (127-312)	0.25
Hepcidin (mg/L)	69 (60-114.1)	144 (72-531)	**0.01**
hsCRP (mg/L)	3.86 (1.95-7.61)	2.58 (1.13-15.17)	0.56
Serum Albumin (g/dl)	4.22±0.30	3.7±0.41	**0.002**
On MHD	3 (11.1%)	39 (72.2%)	**<0.001**

[Table/Fig-1]: Various parameters between responders and poor responders in advanced CKD patient on rHuEPO.
CKD: Chronic kidney disease; SD: Standard deviation; IQR: Interquartile range; eGFR: Estimated glomerular filtration rate; TIBC: Total iron binding capacity; TSAT: Transferrin saturation; hsCRP: High sensitivity C-reactive protein; MHD: Maintenance haemodialysis

	Poor responders (n=54)	Responders (n=27)	Odd Ratio (OR), (significance level)
male	36 (66.7%)	18 (66.7%)	1.03
female	18 (33.3%)	9 (33.3%)	p=0.95
diabetic	25 (46.3%)	11 (40.7%)	1.25
Non diabetic	29 (53.7%)	16 (59.3%)	p=0.63
MHD	39 (72.2%)	3 (11.1%)	20.8
Non MHD	15 (27.8%)	24 (88.9%)	**p<0.001**

[Table/Fig-2]: Association of gender, diabetes and haemodialysis with poor responders compared to responders in advanced CKD patient on rHuEPO (odds ratio).
MHD: Maintenance haemodialysis

DISCUSSION

Hepcidin levels are regulated by iron status and erythropoietic activity [12]. It is now well documented that hepcidin levels are reduced by anaemia and hypoxia while increased by inflammation and decrease eGFR [13-15]. In the present study, it was found hepcidin in MHD patients were higher than non MHD patients. It was supported by other studies [16,17]. The kinetics and technique of haemodialysis may also influence the hyporesponsiveness of EPO [3].

Anaemia management was revolutionised in the late 1980s with the introduction of recombinant human EPO but exogenous substitution in high doses as well as in poor responder is also detrimental leading to stroke, malignancy and precipitation of heart failure.

Present iron indices are not appropriate enough to predict the response of EPO as evident in present study [18]. Hepcidin, a small peptide synthesized mainly in hepatocytes, is the central regulator of systemic iron homeostasis [19]. In the present study, we studied the EPO response in respect of Hb in CKD stage 4 and 5 patients. We defined hyporesponsiveness (poor responder) on the basis of fulfilling the following criteria: haemoglobin less than 10 g/dL, ferritin ≥100 ng/mL, and weekly dose of rHuEPO (Epoetin alfa) 4000 IU. Patients here are of low socioeconomic status and due to unaffordability further dose of rHuEPO was not escalated. Moreover patients are on multiple medications and to add to their misery they have to also afford haemodialysis as required in End Stage Renal Disease (ESRD). Patients on MHD were poor responder which may

be due to increased inflammation as well as extracorporeal blood loss during dialysis and hence ineffective erythropoiesis [20]. Hepcidin was significantly higher in poor responder group than in responder group as it leads to reticulo-endothelial blockade of iron which leads to poor availability of iron for erythropoiesis. The mechanism of hepcidin is by decreasing the level of ferroportin, the major cellular iron transport protein which causes increased intracellular iron stores, decreased dietary iron absorption and decreased circulating iron levels, which may lead to ineffective erythropoiesis. Hepcidin is also determined by stimulation by interleukin 6 which is increased in chronic inflammation [3]. Macrophages are stimulated by inflammation which leads to release of cytokines. Among released cytokines IL-6 is the primary inducer of hepcidin which leads to hypoferremia [21]. The result of present study in regards of hepcidin is supported by some studies [22,23], but not supported by other studies [24]. Inflammatory markers like serum albumin and TIBC were significantly more decreased in poor responder, though serum ferritin and hsCRP was raised significantly in both group. Malyszko J et al., found significant difference of albumin, hsCRP, ferritin but not with hepcidin [25]. The malnutrition-inflammation complex syndrome may also play important role in EPO hyporesponsiveness which may be reflected in the form of decreased serum albumin and cholesterol. Protein-energy malnutrition combined with a state of chronic inflammation may alters the metabolic processes and seriously affects the handling of iron and hence would require complex therapeutic approaches to improve [3]. Drug which inhibit hypoxia induced factor-prolyl hydroxylases (HIF-PH) enzyme, reduces hepcidin with increase in iron mobilization and utilization, as well as increases production of endogenous erythropoietin (EPO) and also overcomes the suppressive effects of inflammation on red blood cell production may be beneficial of such poor responders. For patients on MHD, high convective volumes combined with high permeability membranes which allow an increased clearance of middle-weight molecules, including inflammatory cytokines and peptides which possibly may overcome ESA hyporesponsive [3].

LIMITATION

Limiting factors in present study was due to cross sectional study, small sample size and financial constraints. Further large studies are required in this regard.

CONCLUSION

High hepcidin level may be the cause of hyporesponsiveness to ESA as well an independent bad prognostic marker in anaemic patients of CKD.

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PARTICULARS OF CONTRIBUTORS:
1. Professor Junior Grade and Co-Incharge of Dialysis Unit, Department of Medicine, King George's Medical University, Lucknow, Uttar Pradesh, India.
2. Resident, Department of Medicine, King George's Medical University, Lucknow, Uttar Pradesh, India.
3. Resident, Department of Medicine, King George's Medical University, Lucknow, Uttar Pradesh, India.
4. Associate Professor, Department of Biochemistry, King George's Medical University, Lucknow, Uttar Pradesh, India.
5. Associate Professor, Department of Nephrology, King George's Medical University, Lucknow, Uttar Pradesh, India.
6. Scientist, Department of Toxicology and Experimental Medicine, CSIR-CDRI, Lucknow, Uttar Pradesh, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Satyendra Kumar Sonkar,
Professor, Department of Medicine, King George's Medical University, Lucknow-226003, Uttar Pradesh, India.
E-mail: satyendrakrsonkar@kgmcindia.edu

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