Haematologic Indices and the Response to Erythropoetin Therapy in Chronic Renal Failure

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ABSTRACT

Anaemia has for long been recognized as one of the commonest complications of chronic renal failure. It has been found to significantly influence morbidity; mortality and health related quality of life in these patients. In a bid to further categorize the anaemia in Nigerians with CRF and provide preliminary data on their response to r-HUEPO treatment we conducted this prospective open labelled clinico-pathologic study. Thirty consecutive newly diagnosed CRF patients aged 40.17±13.4 years, and 25 healthy, age and sex-matched controls were studied. The mean creatinine clearance (Cr Cl) was 11.68 ± 9.16 mls/min in the patients and 94.0±16.67mls/min in controls. A comprehensive assessment of their renal status was done as well as full haematological profile including bone marrow aspiration and staining, prothrombin time and bleeding time. Stool test for occult blood and microscopy was done. Urine microscopy, culture and sensitivity was also carried out on all subjects. Subcutaneous recombinant erythropoietin was offered to those that were able to afford it at the dose of 50 units/kg body weight up to a maximum of 4000 units thrice weekly and the response monitored over the study period. We found that anaemia was present in all patients; PCV ranged between 10% and 35% (mean ± SD; 20.27 ±5.6%), in contrast with 37 - 48% (mean ±SD; 41.96 ±16.67%) in normals. PCV correlated positively with creatinine clearance (r=0.97, P =0.0043) and negatively with bleeding time (r=-0.49; P=0.043). The bleeding time was prolonged in 9 (36%) of the patients while it was normal in all controls (P<0.001). Ten Patients had malaria parasitaemia (40%) at presentation when compared with only 3(12%) of the controls (P=0.05). 88% of the patients had normal leucocyte count while all the control subjects had normal leucocyte counts. The platelet counts in the study group ranged between 86 and 246 x 10^9/L while for controls it ranged between 90 and 330 x 10^9/L. Twenty (80%) patients had hypocellular marrow while the remaining 5 (20%) had normocellular marrow. Marrow iron was normal in 12(40%) patients reduced in 11(36.7%) and absent in 7 (23.3%). Recombinant human erythropoietin therapy was effective in 90% of patients that had it. The mean PCV rose from 20.6 ± 3.3 % to 33.6 ± 4.3 % (P=0.008) and retic index from 0.38 to 1.04 over the study period. We conclude that anaemia of increasing severity is common in CRF patients and worsens with progression of the disease; it is hypoproliferative with normochromic normocytic picture in majority of the patients. Contributory factors include iron deficiency, inadequate dialysis dosing, increased bleeding tendencies and megaloblastic anaemia. Erythropoietin therapy was effective but was unaffordable by majority of our patients.

Keywords: *Anaemia, Erythropoietin, Chronic Renal Failure, Response, Haemodialysis.*

INTRODUCTION

Anaemia is the commonest haematological complication of chronic renal failure (CRF) and has been found to contribute significantly to the morbidity, mortality and overall quality of life in these patients[1, 2, 3].

Anaemia commonly accompanies CRF and 67% of patients beginning dialysis in the United States had haematocrit (HCT) of less than 30% while 51%

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had HCT less than 28% [1]. It is symptomatic in about half of end stage renal disease patients worldwide[1] and it is also an independent risk factor for the development of left ventricular hypertrophy (LVH), dilatation, and recurrent congestive cardiac failure[2]. It contribute significantly to cardiovascular disease morbidity and mortality in CRF patients and these have been found to reduce greatly with its treatment[3, 4, 5].

Anaemia in CRF, is usually normochromic normocytic with low reticulocyte index and it usually manifests when clearance falls to less than 40mls/min[6].

Although the aetiology of anaemia in CRF is multifactorial, suppression of erythropoiesis due to reduced erythropoietin production by the kidney has been recognized as the major factor. Other factors include a bleeding tendency due to functional platelet abnormalities, presence of inhibitors of erythropoiesis, shortened red cell survival, and reduced dietary intake and absorption of iron and other haematinics[1, 6]. Other contributory factors include nutritional deficiencies, hookworm infestation, haemoglobinopathies and glucose-6-phosphate dehydrogenase deficiency.

The introduction of recombinant Human erythropoietin (r-HUEPO) into clinical practice has revolutionized the treatment of anaemia in CRF. Erythropoietin (EPO) is vital for the maturation of erythroid progenitor cells as well as terminal maturation of erythroid cells and recombinant preparations are known to be as effective as the natural hormone[7, 8]. It is known to stimulate the maturation of Burst Forming Unit - Erythroid (BFU-E) and Colony Forming Unit - Erythroid (CFU-E), which are erythroid progenitors. CFU-E and mature BFU-E do not survive in the body in the absence of EPO[9]. Apart from correction of anaemia, EPO also have additional therapeutic advantages. These include relief of uraemic signs and symptoms and sexual dysfunction in men, improvement in quality of life particularly vitality and sleep and increased cognitive function. EPO administration is also known to induce reduction in total cholesterol and triglyceride levels as well as reduction in macular edema in diabetic patients[10]. Despite increasing awareness and use of r-HUEPO worldwide, cost considerations has significantly limited its use in this country. Consequently, there is limited experience with this therapy in Nigerians and to our knowledge no published data are yet available on the response of Nigerians with CRF to treatment with r-HUEPO. We therefore undertake this study to:

1. Further categorize the anaemia in Nigerians with CRF and possibly determine the aetiological and other contributing factors.
2. Provide preliminary data on their response to r-HUEPO treatment.

MATERIALS AND METHODS

Thirty consecutive newly diagnosed patients with CRF who satisfied the inclusion criteria were recruited for the study. The inclusion criteria included features of CRF (e.g. nausea, vomiting, pruritus, nocturia, fluid retention) of more than six months duration associated with serum creatinine consistently above 200umol/L and creatinine clearance less than or equal to 40 mls/min, while the exclusion criteria were:

a. Patients on treatment with iron tablets, vitamin B complex or Folic acid
b. Haematologic disorders e.g. Leukaemias, sickle cell anaemia
c. Blood transfusion in the previous 6 months

Twenty-five apparently healthy, age and sex matched subjects who have no laboratory evidence of renal impairment served as the control group. Categorisation of the causes of CRF in the patients were based on validated socio-demographic, clinical, biochemical and imaging criteria used in previous studies[11-14].

Samples were taken to determine serum chemistry including urea, electrolytes, creatinine, proteins, calcium and phosphate. 24-hour urine collection for protein and creatinine clearance was also done. Stool test for occult blood was done in some of the patients after 3 days fasting from meat, fish, vegetables and iron preparation.

Urine microscopy, culture and sensitivity as well as stool microscopy (especially for Hookworm ova) was done on all subjects. Renal Ultrasound Scan was performed on all subjects as well as the following haematological investigations; packed cell \volume (PCV), total/differential leucocyte count, blood film for red cell morphology, platelet count and reticulocyte count. Other haematological investigations included Glucose-6-phosphate dehydrogenase assay, Haemoglobin electrophoresis, bone marrow aspiration and staining for morphology/iron, prothrombin time and prothrombin time ratio and bleeding time. Bleeding time was assessed by Ivy method, while all other haematological investigations were done manually. Ten patients who could afford the cost of r-HUEPO were treated for varying periods at the dose of 50iu/kg body weight up to maximum of 4000units thrice

weekly. The haematological parameters, problems and complications were monitored over the 16-week study period. The ethical committee of the Obafemi Awolowo University Teaching Hospitals complex approved the study.

Statistical Analysis

Data analysis was done with the aid of Statistical Package for Social Sciences 'SPSS' software. Values were expressed as means ± standard deviation (Mean ± SD), independent student t-test as well as Mann Whitney U test for non-parametric data were used for comparison of means while Wilcoxon correlation coefficient was used for determination of associations. P-values of less than 0.05 was taken as statistically significant.

RESULTS

The age range of the patients was 17 - 68 years (mean ± SD; 40.17±13.4 years), while the controls had age range of between 19 and 67years (mean ± SD; 40.24 ± 13.65years) (Table I).

corticomedullary differentiation. Two (7.7%) of the patients had enlarged (Polycystic) kidneys while the remaining three (10%) had normal renal sizes, but poor corticomedullary differentiation and increased echogenicity. The 3 patients with normal renal size had diabetic nephropathy, obstructive uropathy and hypertensive nephrosclerosis respectively.

All the controls had normal renal function, with mean (± SD) serum creatinine and creatinine clearance of 71.36 (± 16μmol/L) and 94.0 (± 16.67) mls/min respectively. All the controls had normal sonographic findings, with mean bipolar diameters of 11.5cm ± 1.02cm.

Haematologic Indices

All the patients studied had anaemia with PCV ranging between 10% and 35% (mean ± SD; 20.27 ± 5.6%) while the controls had normal PCV, ranging between 37 - 48% (mean ±SD; 41.96 ±16.67%).

Table I: Descriptive Statistics of the Study and Control Groups

Parameters	*Patients*	*Controls*	*P-value*
Age	40.17 ±13.44	40.24 ±13.65	0.893
Cr cl (mls/min)	11.68 ± 9.16	111.36 ± 8.02	<0.001
Serum Creat (umol/L)	1247.44±850.22	71.36±16.67	<0.001
Serum Urea(mmol/L)	22.41±10.80	3.67±0.87	0.286
Serum Na^+(mmol/L)	134.76 ± 7.01	132.52 ± 7.66	<0.001
Serum K^+(mmol/L)	5.01 ± 1.3	3.93 ± 0.47	<0.001
Serum HCO_3^-(mmol/L)	19.96 ± 3.20	24.08 ± 2.61	<0.001
PCV(%)	20.27 ± 5.67	41.96 ± 3.13	<0.001
Retics (%)	1.23 ± 0.57	0.84 ± 0.59	<0.001
Retic Index	0.120 ± 0.01	0.84 ± 0.59	<0.001
Platelet(X 10^9/L)	147.48 ± 49.77	215.00 ± 60.48	<0.001
Total WBC (X 10^9/L)	6.65 ± 4.02	3.48 ± 0.99	<0.001
Bleeding Time (min)	10.04 ± 4.67	2.4 ± 0.97	<0.001

All the patients were in established chronic renal failure with creatinine clearance (Cr Cl) that ranged between 1.2 and 32mls/min (mean ± SD; 11.68 ± 9.16 mls/min) (Table I). Seven of them were dialysis dependent hence commenced on maintenance HD on admission. The causes of chronic renal failure in the study group included chronic glomerulonephritis in 12 (40%) of cases; Hypertension in 10 (33.3%) and diabetes in 4 (13.3%). There were also two (6.7%) patients with Polycystic Kidney Disease and aetiology was not known in 2 patients (6.7%).

Twenty-five patients (83.3%) had bilaterally shrunken kidneys, increased echogenicity and loss of

Severe anaemia (PCV <18%) was found in 8(26.6%) of the patients. The remaining 22(73.3%) had mild to moderate anaemia. The degree of the anaemia correlated with the severity of renal impairment. A positive correlation was found between PCV and creatinine clearance "r=0.97 and P =. 0043" (Fig. 1)

The reticulocyte index was low in all the study subjects with the values ranging from 0.05 to 0.5. The reticulocyte counts ranged between 0.1 – 3% with a mean of 1.23 ± 0.57.

The red cell picture was normochromic normocytic in 20 (66.7%) of the patients while 10 (33.3%)

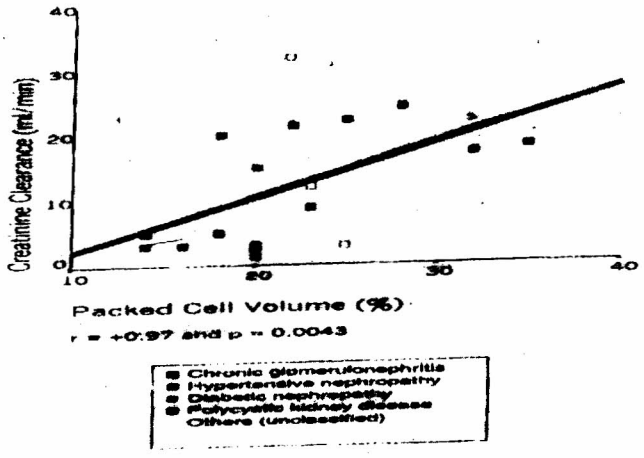

Fig. 1: Correlation between creatinine clearance and PCV

had varying degrees of aniso-poikilocytosis, microcytosis and macrocytosis. All the controls had a normochromic normocytic blood picture.

Malarial parasite was identified in 12 patients (40%) at presentation while only 3(12%) of the controls had malarial parasites on the blood film (P=0.05). Patients with malarial parasites at presentation had lower mean PCV (19.14%) when compared to those who were negative for malarial parasite (PCV of 21.41%) despite higher mean creatinine clearances in the former (13.85 versus 11.25 ml per minute). However, this difference in PCV was not statistically significant (P=0.417).

The WBC count in the study group ranged between 1.9 - 16 x 10^{12}/l (mean ± SD; 6.65 ± 4.02 x 10^{12}/L). Majority of the patients (90%) had normal leucocyte count, while 3 (10%) had leucocytosis. Twenty-two patients (72%) had normal differential count, while 6(24%) had neutrophilia and 1 patient (4%) each had eosinophilia and lymphocytosis. All the control subjects had normal leucocyte counts, ranging between 2.0 x 10^{12}/L and 5.2 x 10^{12}/L (mean ± SD; 3.48±0.99 x 10^{12}/L). The mean WBC count in the study group was higher than the mean WBC count in the control group and the difference was statistically significant (P< 0.05).

The platelet count in the study group ranged between 86 - 246 x 10^9/L (mean ±SD; 147.48 ± 49.77 x 10^9/L), while the controls had platelet counts ranging between 90 and 330 x 10^9/L with a mean of 215 ± 60.48 x 10^9/L. The bleeding time ranged between 3 and 19 minutes in the study group with a mean ±SD of 10.04 ± 4.67minutes. The bleeding time was prolonged in 12 (40%) of the patients while it was normal in all controls (P<0.001). The bleeding time in the controls ranged between 1 and 5 minutes (mean ± SD; 2.40 ± 0.97minutes). A negative correlation was found between the PCV and bleeding time in the study group r=-0.49; P=0.043 (Fig 2). The prothrombin time ranged between 10–13 seconds in the study group with a mean of 11.5 seconds. None of the patients had a clotting abnormality.

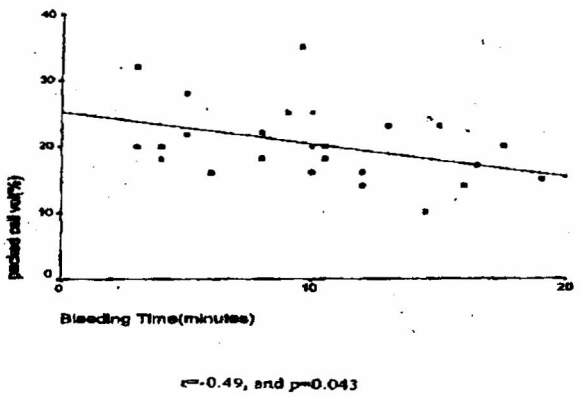

Fig. 2: Correlation between PCV and bleeding time

Two of the patients (6.7%) had haemoglobin genotype 'AS' while the remaining 28 (93.3%) had 'AA'. Amongst the control subjects, one had 'AS' while the remaining 24 had 'AA'. All the patients and controls studied had normal G6PD status.

Bone Marrow Aspiration Results

Bone marrow aspiration findings in the 30 patients studied are as follows: Twenty-three (76.7%) of the patients had hypocellular marrow while the remaining 7 (23.3%) had normocellular marrow. The myeloid erythroid ratio (M:E ratio) was increased in 14 (46.7%) of the patients, reduced in 8 (26.7%), and normal in 8 (26.7%) of the patients.

Erythropoiesis was normoblastic in 19 (63.3%) of the patients, while micronormoblastic erythropoiesis was seen in nine (30%) of the patients. Two patients (6.7%) had megaloblastic changes in the bone marrow. The megakaryocytes were normal in all but one patient with megaloblastic changes who had dysplastic megakaryocytes. Plasma cells were normal in all the patients.

Marrow iron was found to be normal in twelve patients (40%), reduced in eleven patients (36.7%), but absent in seven (23.3%) of the patients.

Infection Screening Results

While all control subjects had essentially normal urine microscopic findings, fifteen (50%) of the patients had confirmed urinary tract infections. *Escherichia coli* , which was the commonest organism isolated was cultured in seven patients. Four patients had *Staphylococcus aureus*, and three other patients had *Klebsiella species* isolated. One patient had mixed infection with *Proteus species* and *Streptococcus fecalis*.

Stool microscopy revealed hookworm, *Ascaris lumbricoides* ova and *Entamoeba hystolitica* in one patient each. The patient with hookworm also had eosinophilia and absent marrow iron. One of the subjects in the control group also had ova of Ascaris in the stool. Stool occult blood was positive in two of the seven patients who had the test.

Response to r-HuEPO Therapy

Only 10 patients were able to sustain r-HuEPO therapy for over 3 months hence the report of response was based on them. They had average of 2000units thrice weekly at onset of treatment but was increased at varying intervals based on individual patient's response to maximum of 4000units thrice weekly in 90% of them. Table 2 shows the mean haematologic parameters in the patients that had r-HuEPO for a maximum 16 weeks. National Kidney Foundation-Dialysis Outcomes Quality Initiative (NKF-DOQI) recommended rise in PCV of 0.6 to 1.8% per week [15] and the target PCV of 33% was achieved in 7 (70%) of the patients. Initial rise in PCV was also satisfactory in 20% of the patients but later reached a plateau while the response was poor in the last patient (10%). The increase in PCV was with a trend towards significance at week 6 (P=0.056) but was statistically significant at weeks 10 and 16 with P-values of 0.032 and 0.008 respectively. As expected the reticulocyte count increased with rise in PCV in all patients who had r-HuEPO therapy. There was also statistically significant reduction in bleeding time in all patients who were treated with r-HuEPO. Figure 3 shows the response of the patients to r-HuEPO therapy.

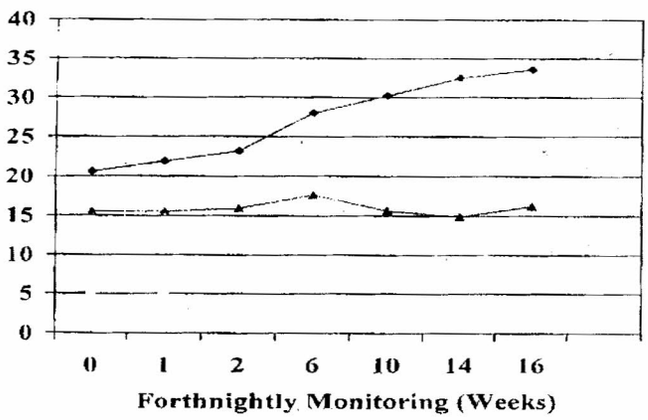

Fig. 3: Haematological response to r-HuEPO therapy

Table 2: Mean Haematologic Parameters In patients Managed with r-HuEPO

PARAMETERS	Pre- Treatment (Week 0)	WEEK 1	WEEK 2	WEEK 6	WEEK 10	WEEK 14	WEEK 16
PCV (%)	20.6	21.9	23. 2	28	30.2	32.5	33.6
WBC (X 10^9 cells/L)	5.42	5.34	5. 72	6.01	6.57	11.65	7.26
Platelets (X 10^9 cells/L)	155.00	155.20	159. 00	175.75	156.00	149.00	162.40
Reticulocyte Index	0.38	0.43	0. 60	0.76	0.83	2.00	1.04
Bleeding time (min)	8.4	9.38	8. 20	8.00	7.00	7.10	7.10

		P-value
PCV pre-treatment Vs PCV Week 2	-	*0.310 ns*
PCV pre-treatment Vs PCV Week 6	-	*0.056 ns*
PCV pre-treatment Vs PCV Week 10	-	*0.032 s*
PCV pre-treatment Vs PCV Week 16	-	*0.008 s*
PCV Week 2 Vs PCV Week 10	-	*0.056 ns*
PCV Week 2 Vs PCV Week 16	-	*0.008 s*

DISCUSSION

Anaemia is of considerable importance in CRF patients as its treatment has been shown to lead to improvement in health related quality of life, reduction in frequency of hospitalization, morbidity and overall mortality profile[3, 15, 16]. Anaemia also contributes significantly to the burden of cardiovascular disease, which is now recognised to be the commonest cause of death in CRF patients.

This study, in agreement with the finding of Oluboyede *et al* [17] found varying degrees of anaemia in all the patients underscoring the magnitude of the problem. In an earlier report from our centre 72% of the recruited patients were anaemic[18], this may be explained by the differences in the severity of CRF in the patients in the different studies.

The severity of anaemia in this study correlated with the severity of renal failure as in previous studies[15, 19]. This may be a reflection of progressive decline in erythropoietin secretion as well as increasing levels of uraemic inhibitors of erythropoiesis as renal function deteriorates. In agreement with previous studies, the peripheral blood picture was normochromic normocytic in majority of the patients in this study[20].The proportion of patients with iron deficiency anaemia confirmed by absent marrow iron is in keeping with the observation by Silverberg *et al* [2] and Oluboyede *et al* [17].

The low reticulocyte index in all the patients in this study categorizes the anaemia as hypoproliferative. The fact that majority of the patients (92%) studied had normal reticulocyte count despite low reticulocyte index underscores the importance of using the reticulocyte index rather than the reticulocyte count in patients who have anaemia. The finding of low to normal reticulocyte count in this study is in keeping with the findings of others[18].

Normal WBC count was found in majority of the patients in this study in agreement with others[18, 20]. This is because the effect of uraemia on the leucocyte series is mainly functional and in fact some authors suggest that there is a poor leucocyte response to infection or indeed some degree of suppression of granulopoiesis in CRF[20, 21]. The higher incidence of malaria at presentation in the patients compared to the controls was not statistically significant hence malaria infection could not be labelled as a confounding factor for anaemia in CRF.

The finding of normal platelet counts in all the patients is in agreement with our earlier finding[20] and that of others[22, 23]. The effect of uraemia on the platelets is mainly functional and manifests as prolonged bleeding time leading to bleeding tendencies[22, 23, 24]. Prolonged bleeding time was indeed found in 36% of the patients in this study. The positive correlation found between the serum creatinine and bleeding time implies that the platelet dysfunction worsens with worsening renal function. A complex platelet dysfunction has been described in CRF patients and worsening of these functional abnormalities with progressively declining renal function is the likely reason for this correlation[22, 23, 24]. However platelet functional abnormalities are not the only factors involved in the pathogenesis of the increased bleeding tendency in uraemia. Anaemia also influences the bleeding tendency by negatively affecting the rheological component of the interaction between platelets and vessel wall [24]. The negative correlation between PCV and bleeding time in this study supports this assertion.

Majority (77%) of the patients in this study had hypocellular marrow unlike 19.4% found in the study by Akinsola *et al* [15] though similar to the findings of Oluboyede *et al* [17]. The suppression of cellular components in the bone marrow of the patients in this study affected the erythroid cells more than other cell lines as evidenced by increased M: E ratio in majority of the patients.

Erythropoietin therapy represents one of the most important advancements in management of CRF patients in the last 2 decades. Its use in CRF patients in Nigeria has been limited by its exhorbitant cost hence paucity of data [25]. In fact a recent Medline search did not reveal any such documentation hence our decision to document our preliminary findings on the responsiveness of ten anaemic CRF patients to r-HuEPO therapy. A randomised controlled study in a larger population of CRF patients is being proposed and shall commence soon.

Recombinant human erythropoietin was largely unaffordable by majority of our patients. It was beneficial in majority of the patients given as the NKF-DOQI recommended rise in PCV of 0.6 to 1.8% per week [16] and the target PCV of 33% was achieved in 70% of the patients. Erythropoietin therapy is known to improve haematocrit, appetite, sexual and cognitive functions. Other beneficial effects include regression of left ventricular hypertrophy, retardation of progression of kidney disease as well as significant improvements in the survival and overall quality of life in CRF patients[5, 26, 27]. Some of the problems of Erythropoietin use include its worsening of hyperten-

sion through stimulation of endothelin release and the dreaded pure red cell aplasia (PRCA) thought to be secondary to development of erythropoietin-induced antibodies [28].

Initial rise in PCV was satisfactory in 20% of the patients but later reached a plateau probably as a result of under dosing and iron deficiency in 1 patient and inadequate dialysis in the other. The response was poor in the last patient because of septicaemia and inadequate dialysis. These factors are recognised non-erythropoietin related factors that are known to reduce responsiveness to erythropoietin[21]. Others include occult blood loss, folate and vitamin B12 deficiencies, infection or infestations, inflammation, bone marrow suppression and hyperparathyroidism. Marrow fibrosis, aluminium toxicity, carnitine deficiency, intrinsic red cell defects like haemoglobinopathies and bone marrow disorders are also known causes of erythropoietin resistance[29].

As expected the reticulocyte count increased with rise in PCV in all patients who had r-HuEPO therapy. The increase in PCV was statistically significant and there was statistically significant reduction in bleeding time in all patients who are treated with r-HuEPO.

As r-HuEPO was largely unaffordable by majority of our patients, we opine that a reduction in the cost of r-HuEPO would enable more patients to benefit from its use and consequently its beneficial effects.

In conclusion, anaemia of increasing severity is common in CRF patients and worsens with progression of the disease; the anaemia is hypoproliferative with normochromic normocytic picture in majority of the patients. A sizable proportion of anaemic CRF patients have iron deficiency anaemia and other recognized contributory factors include bleeding tendencies and megaloblastic anaemia. Bleeding tendency, which correlates with the degree of renal impairment and the degree of anaemia is common in patients with CRF.

r-HuEPO therapy was effective in majority of the patients and causes of r-HuEPO hyporesponsiveness include iron deficiency, inadequate dialysis and underdosing due mainly to financial constraint. Early detection and treatment of anaemia in CRF patients should be aggressively pursued as this has been established to lead to reduction in morbidity and mortality and consequently increased quality of life and life expectancy.

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