Effect of Dialysis on Erythropoietin and some Hematological Parameters in Patients with Chronic Renal Failure

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ABSTRACT

Background and Objectives: A prospective study was carried out for estimation the concentration of the erythropoietin hormone and its effects on some hematological parameters in chronic renal failure patients despite medical treatment. The object of the study was to evaluate the available evidences which support the relationship between anemia and adverse outcomes in chronic renal failure patients, to estimate the erythropoietin concentration of plasma in chronic renal failure patients and to examine the relationship between the anemia and erythropoietin concentration in patients with chronic kidney disease.

Methods: One hundred and eleven (111) patients with CRF on dialysis in the Dialysis and Kidney Disease Center in Hawler Teaching Hospital and (40) control groups were studied. Haematological parameters: were performed by conventional manual methods. The quantitative measurement of erythropoietin concentration in serum was done by using the Erythropoietin ELISA kit (IBL Immuno-Biological Laboratories Hamburg Erythropoietin Kit, German).

Results: Hemoglobin, red blood cells, packed cell volume, and mean corpuscular hemoglobin were significantly decreased ($P<0.001$) in all age groups of Chronic renal failure patients, while, erythrocyte sedimentation rate and mean corpuscular volume (MCV) were increased significantly ($P<0.001$). Total white blood cells were decreased significantly ($P<0.01$) at the ages (40-60, >60) years groups, while not significantly decreased at the ages (<20, 20-40) years. Plasma erythropoietin was decreased significantly at the level ($P<0.001$) in chronic renal failure patients.

Conclusions: Anemia is a common and often an early complication of chronic renal diseases and decreased renal production of erythropoietin is the major cause of anemia in these patients.

Key words: Dialysis, Erythropoietin, Chronic renal failure.

INTRODUCTION:

Chronic kidney disease (CKD) is characterized by progressive deterioration of kidney function, which led eventually into a terminal stage of chronic renal failure (CRF). End-stage renal disease (ESRD) represents the total inability of kidney to maintain homeostasis, thus, at this stage it is necessary to use treatment methods that substitute kidney function to maintain good health and life. These methods tation[1]. Anemia has been recognized as one of the most common problems causing morbidity in pre-dialysis patients and in patients with chronic kidney disease (CKD), while they are on dialysis. The Reduction of the functioning renal tissue in CKD decreases the kidney's ability to respond to reduced oxygen delivery and to produce erythropoietin (EPO), a hormone that promotes red blood cell (RBC) production in the bone marrow. The primary cause of anemia in the CRF

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the diseased kidneys. Additional factors that may contribute to anemia are short RBC survival time, iron and folate deficiencies, and inhibition of haematopoiesis by toxic metabolites or inhibitors[2,3]. Human EPO is a glycosylated protein hormone with molecular weight 30.400 KDa. The native compound is composed of 166 amino acids, although in the circulating form there are only 165 amino acids and four carbohydrate groups[4,5]. The cellular site of EPO synthesis and release is the peritubular fibroblast cells located in the renal cortex [6]. However, other sites of EPO expression have been reported, including liver (in fetal stage) spleen, lungs [7,8], bone marrow macrophages, early colony-forming cells, umbilical cord monocytes differentiated into a macrophage phenotype in vitro, and brain astrocytes[9]. Erythropoietin promotes the proliferation of erythroid progenitor cells, maintains their survival, and facilitates their differentiation. Hypoxia is the only physiologic stimulus that increases erythropoietin [10].

MATERIALS AND METHODS:

One hundred and eleven (111) patients with CRF on dialysis in the Dialysis and Kidney Disease Center in Hawler Teaching Hospital and (40) control groups were studied. Patients and controls were divided into four age groups (with 20 years intervals). Samples of blood were collected and divided in to two tubes (each 2 ml), both collected into anticoagulant ethylene diamine tetra acetic acid (EDTA). One of them for estimation of plasma erythropoietin, and the other for hematological parameters. Plasma samples were separated and stored at freezing point -84 C for further investigations until assay.

1. Determination of EPO: The Erythropoietin ELISA kit (IBL Immuno-Biological Laboratories Hamburg Erythropoietin Kit, German) provides method type assay erythropoietin is first bound by a rabbit anti-EPO -antiserum immobilized on the solid phase of amicro -titer-plate. After the washing step a second antibody to erythropoietin from rabbit conjugated to biotin forms a sandwich complex with EPO. After incubation with an anti-biotin antibody conjugated to alkaline phosphatase and addition of a substrate P--nitrophenyl phosphate (PNPP) a yellow color is formed which is proportional to the concentration of erythropoietin. On a semi logarithmic graph paper the concentration of the standards (x-axis, logarithmic) were plotted against their corresponding optical density (y-axis, linear). Alternatively, the optical density of each standard and sample can be related to the optical density of the zero standard, expressed as the ratio OD/ ODmax, and then plotted on the ordinate.

2.Haematological parameters: were performed by conventional manual methods.

Statistical analysis

The statistical analysis of results of this study was performed by using statistically available software (Stat graph Version 5 and SPSS Version 11.5).by using T-test and the Pearson correlation method. P< 0.05 was considered statistically significant.

RESULTS:

Plasma erythropoietin in patients with CRF and controls at different ages: The Elisa test result of EPO shows that there were significant decrease in EPO in patients with CRF (0.6767± 0.2590) mIU/ml when compared with control groups (54.780±5.2802) at the level (P<0.001).

Hematological parameters at different age groups:

(Table1) shows the effect of CRF on hemoglobin (Hb), packed cell volume (PCV), erythrocyte sedimentation rate (ESR), white blood cell count(WBC), red

cell indices (MCV,MCH and MCHC) compared with controls at the different ages. Blood smears of most of these should red cells with anisocytosis; microcytic in some patients, while normocytic or macrocitic in others.

Table (1): Hematological parameters changes in patients with CRF compared to the control group.

Parameters	Patient (N= 111)	Controls (N=44)	Statistical evaluation
Hb (gm/dl)	8.6870 ±0.1551	13.610±0.1141	P<0.001
PCV %	27.891 ±2.6421	40.700±0.5590	P<0.001
RBC (cell/mm³)x 10^6	3.0470 ±0.0511	4.8000 ± 0.0600	P<0.001
ESR (mm/hr)	66.922 ±2.5211	8.0500±1.2721	P<0.001
WBC (cell/mm³) x10^3	6.4000±0.6000	5.9002±0.1871	N.S
Retic %	1.2002±0.0510	1.0800±0.0700	N.S
PLT (cell/mm³)x10^3	198.00±2.8650	196.77±4.9941	N.S
MCV(fL)	90.530 ± 0.6000	84.860±0.9553	P<0.001
MCH (pg)	28.110±0.2493	28.440±0.2823	N.S
MCHC(gm/dl)	31.050±0.1861	33.571 ±0.2710	P<0.001

There was a significant decrease in (Hb) level between patients (8.6870 ±0.1551) and controls (13.610±0.1141) (P<0.001), (PCV) between patients (27.891 ±2.6421) and controls (40.700±0.5590) (P<0.001),(RBC) between patients (3.0470 ±0.0511) and controls (4.8000 ± 0.0600) (P<0.001), and (MCHC) of patients (31.050±0.1861) and controls (33.571 ±0.2710) (P<0.001). There was a significant increase in (ESR) between patients (66.922 ±2.5211) and controls (8.0500±1.2721) (P<0.001), (MCV) between patients (90.530 ± 0.6000) and controls (84.860±0.9553) (P<0.001). There was no significant difference in (WBC), (Retic.), (PLT) and (MCH) between patients and controls.

Hematological parameters at the ages <20:

Table (2) shows the statistical evaluation of haematological parameters according to the analytical t-test, the Hb, PCV, RBC, ESR, WBC, Retic., PLT and MCV, MCH and MCHC at the age group <20 years. There was a significant decrease in (Hb) between patients of this age group (8.6380 ± 0.3471) and controls (13.862±0.0972)(P<0.001), (PCV) between patients (28.990 ±1.1541) and controls (40.250 ± 0.7733)(P<0.001), (RBC)between patients (3.0731 ±0.1130) and controls (4.9000± 0.0849)(P<0.001) and (MCHC)between patients (30.080 ±0.586) and controls (34.510 ±0.5553) (P<0.001). In contrast there was a significant increase in (ESR)between patients (56.383 ±4.3941) and controls (4.8750 ±1.0762)(P<0.001) and (MCV) between patients (90.723± 1.4690) and controls (82.267 ±1.1860)(P<0.001). No significant difference was seen regatding (WBC),(Retic.),(PLT), and(MCH)between the two groups.

Table (2): Hematological parameter changes in patients with CRF at the age groups of < 20 years.

Parameters	Patients (N=21)	Controls (N=8)	Statistical evaluation
Hb (gm/dl)	8.6380 ± 0.3471	13.862±0.0972	P<0.001
PCV %	28.990 ±1.1541	40.250 ± 0.7733	P<0.001
RBC (cell/mm³)x10⁶	3.0731 ±0.1130	4.9000± 0.0849	P<0.001
ESR (mm/hr)	56.383 ±4.3941	4.8750 ±1.0762	P<0.001
WBC (cell/mm³)x10³	6.0800± 0.5125	6.2000±0.7904	N.S
Retic %	1.0803±0.137	1.135±0.1942	N.S
PLT (cell/mm³)x10³	192.76±10.7812	189.12±10.563	N.S
MCV(fL)	90.723± 1.4690	82.267 ±1.1860	P<0.001
MCH (pg)	27.468±0.7000	28.350±0.5500	N.S
MCHC(gm/dl)	30.080 ±0.586	34.510 ±0.5553	P<0.001

Hematological parameters at the ages 20-40:

(Table 3) shows the statistical evaluation of hematological parameters according to the analytical t-test at 20-40 years age interval. There was a significant decrease in (Hb)between patients (8.1590± 0.2695) and controls (14.018 ±0.2490) (P<0.001), (PCV)between patients (26.259 ±0.7960) and controls (42.545 ±0.3480)(P<0.001), (RBC) between patients (2.9140 ±0.0763) and controls (4.9550±0.1053)(P<0.001) and (MCHC) between patients (30.737±0.3485) and controls (33.067±0.5000) (P<0.001). There was a significant increase in (ESR) between patients (66.330 ±3.6081) and controls (4.8189 ±1.0764) (P<0.001) and (MCV) between patients (89.390±1.5390) and controls (85.886±1.7200)(P<0.001). No significant differences in (WBC), (Retic.), (PLT), and (MCH) between patients and controls.

Hematological parameters at the ages 40-60:

(Table 4) shows the statistical evaluation according to the analytical t-test for age interval. There was a significant decrease in (Hb) between patients (8.8800±0.2612) and controls (13.320±0.1712) (P<0.001), (PCV) between patients (28.159±0.8580) and controls (40.300±1.0430)(P<0.001), (RBC) between patients (3.0900±0.0964) and controls (4.7502±0.1290) (P<0.001), (MCHC) between patients (31.385±0.1977) and controls (33.164±0.5164)(P<0.001) and (WBC) between patients (5.2594±0.2200) and controls (6.9099±0.5404) (P<0.01). There was significant increase in (ESR) between patients (69.655±4.8944) and controls (10.500±1.0573)(P<0.001) and (MCV)between patients (90.990±0.9141) and controls (85.280±2.7480)(P<0.001). No significant differences in (Retic.), (PLT), and (MCH) between patients and controls at these ages were found.

Table (3): Hematological parameter changes in patients with CRF at the age groups of 20-40 years.

Parameters	Patients (N= 27)	Controls (N= 11)	Statistical evaluation
Hb (gm/dl)	8.1590± 0.2695	14.018 ±0.2490	P<0.001
PCV %	26.259 ±0.7960	42.545 ±0.3480	P<0.001
RBC (cell/mm³) x10⁶	2.9140 ±0.0763	4.9550±0.1053	P<0.001
ESR (mm/hr)	66.330 ±3.6081	4.8189 ±1.0764	P<0.001
WBC (cell/mm³) x10³	5.6100± 0.3101	6.1001±0.4241	N.S
Retic %	1.0913±0.1000	1.0501±0.1304	N.S
PLT (cell/mm³) x10³	195.14±4.6433	188.27±2.7200	N.S
MCV(fL)	89.390±1.5390	85.886±1.7200	P<0.001
MCH (pg)	27.630±0.6000	28.336±0.3795	N.S
MCHC(gm/dl)	30.737±0.3485	33.067±0.5000	P<0.001

Table (4): Hematological parameter changes in patients with CRF at the age groups of 40-60 years.

Parameters	Patient (N= 44)	Control (N= 10)	Statistical evaluation
Hb (gm/dl)	8.8800±0.2612	13.320±0.1712	P<0.001
PCV %	28.159±0.8580	40.300±1.0430	P<0.001
RBC (cell/mm³) x10⁶	3.0900±0.0964	4.7502±0.1290	P<0.001
ESR (mm/hr)	69.655±4.8944	10.500±1.0573	P<0.001
WBC (cell/mm³) x10³	5.2594±0.2200	6.9099± 0.5404	P<0.01
Retic %	1.2862±0.0960	1.2703±0.1202	N.S
PLT (cell/mm³) x10³	200.59±4.0000	205.70±12.032	N.S
MCV(fL)	90.990±0.9141	85.280±2.7480	P<0.001
MCH (pg)	28.459±0.3290	28.210±0.7683	N.S
MCHC(gm/dl)	31.385±0.1977	33.164±0.5164	P<0.001

Hematological parameters at the ages >60: (Table 5) show the statistical evaluation according to the analytical t-test for hematological parameters at the ages >60 years. There was a significant decrease in (Hb) between patients (9.0363±0.3662) and controls (13.290±0.2352)(P<0.001), (PCV).between patients (28.467±1.1352) and controls (39.545±1.1854)(P<0.001), (RBC) between patients (3.1000±0.1172) and controls (4.6321±0.1341) (P<0.001), (MCHC) Between Patient (31.830±0.4963) and controls (33.770±0.5542) (P<0.001) and (WBC) between patients (4.5221±0.1919) and controls (5.4427±0.3041) (P<0.01).There was a significant increase in (ESR) between patients (73±5.8300) and controls (11.360±1.3716) (P<0.001) and (MCV) between patients (90.860±0.8374) and controls (85.360± 1.1783) (P<0.001). No significant differences in (Retic.) (PLT) and (MCH) between patients and controls at this age group were found.

Table (5): Hematological parameter changes in patients with CRF at the age groups of > 60 years.

Parameters	Patient (N= 19)	Control (N= 11)	Statistical evaluation
Hb (gm/dl)	9.0363±0.3662	13.290±0.2352	P<0.001
PCV %	28.467±1.1352	39.545±1.1854	P<0.001
RBC (cell/mm³) x10⁶	3.1000±0.1172	4.6321±0.1341	P<0.001
ESR (mm/hr)	73±5.8300	11.360±1.3716	P<0.001
WBC (cell/mm³) x10³	4.5221 ± 0.1919	5.4427± 0.3041	P<0.01
Retic %	1.3061±0.1312	1.0900±0.1512	N.S
PLT (cell/mm³) x10³	201.89±3.6411	201.63±6.0790	N.S
MCV(fL)	90.860±0.8374	85.360± 1.1783	P<0.001
MCH (pg)	28.700±0.4333	28.815±0.5577	N.S
MCHC(gm/dl)	31.830±0.4963	33.770±0.5542	P<0.001

DISCUSSION:

The present study showed that in patients with CRF, EPO secretion was relatively reduced compared with blood Hb concentration. Such finding was seen by others[12]. The reasons for decreased EPO production in the diseased kidneys are explained primarily by destruc-tion of the EPO-producing fibroblasts of the kidney interstitial fibrosis and the overall reduction in renal mass[13]. Normally the response to EPO is an increase in the number of reticulocytes to more than 2.5 %[14], whereas, our results showed non-significant increase in reticulocyte and this may confirm the fact that there is no well response to EPO in these patients. Evidences for resistance to EPO is either failure to achieve the target Hb concentration while receiving more than

or a continuous need for such dosage to maintain the target. The most common cause for incomplete response to EPO is iron deficiency[15]. Also these patients often have been infected or have had surgery, leading to an inflammatory block evidenced by elevation in ESR and therefore rHu-EPO hyporesponsiveness[16,20]. The elevated ESR in patients with CRF is mostly due to inflammation and infection, which underlies most of the vascular disease and catabolic processes occurring in CKD and CRF as a cause &/or sequels, which are most probably due to elevated circulat-ing levels of inflammatory cytokines such as interleukin-6[22]. Also failure to respond to rHu-Epo during treatment of CRF also may be due to enhanced immune activation, which is known to occur in renal failure patients [21]. Deficiency of the EPO is the main cause of the progressive decline in Hb concentration, PCV and RBC which occur frequently in patients with CRF. Erythropoietin stimulates terminal differ-entiation of committed erythroid progenitors in the bone mar-row [17]. Inhibitors for red blood cells production have also been described in uremia [18], inhibition of growth of erythroid progenitor cells, including CFU-E and BFU-E, or inhibition of heme synthesis through blocking the EPO-specific receptors on these cells[19].

We found distinct negative correlation between endogenous EPO levels, reticulocyte number and WBC count, this can be explained by the observation that EPO is present in non-erythroid blood lines including myeloid cells, lymphocytes and megakaryocytes and other progenitor include CFU-E and CFU-B [23,24]. Since MCH is an accurate early marker of iron deficiency anemia. Anemia in CRF can not be explained by iron deficient erythropoiesis only, since MCH remained normal. The changes in MCV and MCHC predominantly occurred during the period of CRD; therefore, the explanation of this change may be due to EPO deficiency by diseased kidney. The decrease in MCHC evidenced by the presence of microcytic mia, while macrocytosis is caused by EPO deficiency in some other patients. The unchanged MCV in some patients is explained by the concomitant occurrence of both iron deficiency anemia and EPO deficiency. These findings were also experienced by other authers[25,26].

CONCLUSIONS :

From this study we can conclude that anemia is a common and often early complication of CRD and that deficient renal production of EPO is the major cause of anemia in these patients in addition to iron deficiency.

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