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ERYTHROPOIETIN AND ANEMIA IN CHRONIC RENAL FAILURE

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ABSTRACT

Serum erythropoietin levels were measured by radioimmunoassay and compared to the severity of anemia in patients with end stage renal disease of different etiology, on chronic hemodialysis. It was demonstrated that the difference in severity of anemia in those patients is a consequence of the difference in erythropoietin production, and it was stressed that in patients with polycystic kidney disease the kidney tissue kept its endocrine function although had no residual excretory renal function. The positive correlation between hematocrit values and erythropoietin levels indicates that in these patients erythropoietin synthesis is not regulated by general hypoxia. It is suggested that control of erythropoietin production in diseased kidney differs from the physiologic one.

Anemia in chronic renal failure is generally assumed to be the consequence of low level of erythropoiesis which cannot cope with shortened red blood cells life span, iron deficiency, blood loss, or the effect of possible inhibitors. Long term maintenance of patients on chronic hemodialysis and the development of a sensitive radioimmunoassay for human erythropoietin (Ep) allow a more detailed study of the anemia in chronic renal insufficiency.

The aim of this study was to evaluate the significance of Ep levels and the presence of erythropoiesis inhibitors in different kidney disease for the severity of anemia.

PATIENTS STUDIED AND METHODS

Seventeen patients undergoing maintenance hemodialysis at the City Hospital of Beograd were investigated. The etiology of end stage renal disease in 8 patients was polycystic kidney disease (Group I) and kidney disease (glomerulonephritis or pyelonephritis) in 9 patients (Group II). Glomerulonephritis was diagnosed on kidney biopsy specimens while polycystic kidney disease and pyelonephritis were diagnosed by means of X-ray, scintigraphy and echotomography. All patients were dialyzed 3 times a week for 5 hours with a cuprophane membrane of $1m^2$. The blood flow rate was 200 ml/min and dialyzate flow was 500 ml/min. All patients with the exeption of patient #3 and #8 were on dialysis for more than one year (12 to 120 months). Residual creatinin clearance was less than 1 ml/min in all patients. Predialysis blood samples for the study were obtained immediately before regular hemodialysis. Blood was centrifuged and serum samples were kept frozen at -20°C until tested. No patient was studied within 30 days following a transfusion. All the patients had given their consent before the study.

As can be seen from Table 1, body weight, blood urea and creatinine

values, plasma proteins and transferin, as a measure of state of nutrition, for each group of patients studied were similar.

Patients were essentially without residual kidney function and were on dialysis under the same conditions. The only difference was, besides etiology of end stage renal disease, the severity of anemia. Hematocrit values were 0.38 ± 0.07 in the first and 0.19 ± 0.03 in the second group of patients.

Serum Ep levels were measured by the previously described radioimmunoassay technique for Ep by Garcia et al. (1) and by measuring 48 hours ^{59}Fe incorporation in CBA mice with posthypoxic erythrocytosis injected 2 x 0.5 ml sera on the 6th and 7th day posthypoxia (2).

The presence of possible serum inhibitors were determined in mouse bone marrow CFU-E assay as suggested by Wallner et al. (3). The methylcellulose culture technique (4) as described elsewhere (5) was used. Briefly the culture mixture consisted of 0.8% methylcellulose, 30% fetal calf serum, alphathioglycerol 10^{-4}M, Dulbecco's minimum essential media and Ep 0.25 U. Plasma from mice previously irradiated and injected with phenylhydrazine was the source Ep (6) with Ep levels of about 10 U/ml. The mixture containing 10^5/ml bone marrow cells was plated in 35 mm plastic tissue culture dishes (four dishes for each experimental group). Five or 10% of patients sera sterile filtered was added to the plates. Normal human serum was used as a standard control. The plates were incubated at 37°C in a humidified atmosphere with 5% CO_2 in air for 48 hours. Patients sera and controls were tested on the same cell suspension and the experiment was repeated twice. The results of CFU-E inhibition are presented as a percentage of the control number expressed as 100%.

RESULTS

The results presented in Table II demonstrate that the severity of anemia in patients with end stage renal disease differs depending on the etiology of the kidney disease. Patients with polycystic kidney disease had mild anemia or were not anemic at all (Group I) contrary to the patients with other kidney diseases (Group II) who were anemic and transfusion-dependent. Ep levels were increased in 6 out of 8 patients from Group I. One of the low values in this group was found in a patient whose hematocrit was higher than normal (Patient 6). The mean Ep value in Group I was statistically higher than in Group II and in some patients was high enough to be measured in the rather more insensitive mouse bioassay. Ep levels in patients from Group II were low except in one patient (No. 11). This patient had chronic pyelonephritis diagnosed 15 years earlier and was treated with regular hemodialysis for 40 months. Subsequent ecnosonography and computerized tomography of the abdomen revealed in this patient secondary cysts in both kidneys. Therefore the patient had to be excluded from Group II, although the patient was anemic at the time of the study.

When serum Ep levels were presented as a function of the hematocrit values (Fig. 1), a positive correlation between erythropoietin and hematocrit was found. Ep level determinations in the same assay in the sera of healthy persons and patients with iron deficiency anemia are given in Fig. 2 for comparison. A negative correlation between Ep level and hematocrit values was demonstrated.

Serum inhibitors determined in vitro were found in all but one serum tested. As can be seen from Table III the percentage of inhibition varied from 55% to 95% when 10% of patients sera was added to mouse bone marrow cultures. No inhibition was detected when the same amount of normal sera was added to the

system. The inhibitory effect of sera from Group I and II was similar. In Table III only the results obtained in one experiment are presented, since very similar results were obtained when the same experiment was repeated.

DISCUSSION

Two groups of patients with kidney disease and end stage renal failure were studied in order to determine whether the difference in the severity of anemia is due to the difference in Ep levels. Particular care was taken to compare patients of similar age and duration of maintenance on chronic hemodialysis, similar body weight and state of nutrition. Patients were essentially without residual kidney function and were dialyzed under the same conditions. The only difference between the two groups, besides ethiology of renal failure, was the difference in the severity of anemia.

The results presented indicate that the difference in the severity of anemia in those patients with chronic renal insufficiency is a consequence of the difference in Ep production in two groups of patients studied, since higher Ep levels were found in patients with polycystic kidneys and a positive correlation between Ep and hematocrit was found. Serum Ep level was determined by radioimmunoassay which is not influenced by enhancing or inhibiting factors (1) and has been shown to detect biologically active Ep in human serum (7). However, in patient 11 anemia was present in spite of increased Ep level demonstrated by radioimmunoassay and not detected in a bioassay. It can be suspected, as demonstrated by Sherwood and Goldwasser (8), that in the serum of this patient substance immunologically reacting as Ep but devoid of biological activity was detected by radioimmunoassay.

Chandra (9) also have reported higher levels of Ep in patients with polycystic kidney disease as compared with other hemodialysis patients; but our

results differ from theirs in that we found higher Ep levels, although the same radioimmunoassay was used. This is also in accordance with higher hematocrit values in the polycystic kidney disease patients we have studied, as compared to their patient population. Nevertheless, both studies speak in favor of the conclusion that the less severe anemia in polycystic kidney patients is primarily due to higher Ep levels.

It is of interest to stress that although the patients from both groups did not have any residual excretory renal function, the kidney tissue in patients with polycystic kidney disease kept its endocrine function. This conclusion seems to be reasonable, although from the results we have obtained it cannot be distinguished whether Ep in serum is of renal or extrarenal origin. The conclusion that diseased kidney can sustain secretion of Ep was demonstrated by Dagher et al. (10) in patients with kidney allograft in whom Ep levels were higher in renal venous plasma from the original diseased kidney than in the transplanted one. This idea is also supported by erythrocytosis developing during maintenance hemodialysis therapy in patients with end stage renal disease (11) and by the difference in Ep levels between uremic nephric and anephric patients found by Caro et al. (12).

The positive correlation between the hematocrit values and Ep levels we have found indicates that in those patients Ep synthesis is not regulated by general hypoxia. In patients with polycystic kidney disease, the stimulation of Ep production can be explained to be due to the local hypoxia of remnant kidney tissue by the pressure of cystic formation. However a positive correlation between hemoglobin concentration and Ep levels in other dialysed patients who were transfusion independent was found by DeKlerk et al. (13) as well and also by Radtke et al. (14) in patients on chronic hemodialysis. Deficient feedback regulation of Ep in kidney transplant patients with

polycythemia was studied by Thevenod et al. (15), and it was found that transplanted kidneys operate within the normal feedback regulation while the native diseased kidneys do not. It could be suggested therefore that control of Ep production in diseased kidney differs from the physiologic one.

The role of inhibitors of erythropoiesis in anemia of chronic renal failure is not completely clarified. McGonigle et al. (16) have found that serum inhibitors of erythropoiesis determine the degree of anemia in patients with renal failure. However, Mladenovic et al. (17), using an autologous sheep model, could find no significant inhibition of uremic sheep serum in vitro, and their results suggest that uremic toxins are not a major contributing factor in end stage renal disease. We have not found any correlation between hematocrit values and percent of inhibition of CFU-E derived colonies in vitro in the sera of studied patients. This is in agreement with our first conclusion and findings of Chandra et al. (9) that the level of erythropoiesis in patients with end stage renal failure depends predominantly on the availability of Ep. Replacement therapy with recombinant Ep in patietns with advanced kidney disease in which hemodialysis replaces the failing excretory function, besides benefiting the patients, will prove that anemia is of Ep privation.

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FIGURE LEGENDS

Fig. 1. Correlation between immunoreactive erythropoietin levels and hematocrit (PCV) values in patients with renal failure maintained on chronic hemodialysis (Patient No. 6 excluded from calculation of correlation coefficient). $r = + 0.5330$, $p < 0.05$.

Fig. 2. Correlation between immunoreactive erythropoietin levels and hematocrit (PCV) values in healthy persons and patients with iron deficiency anemia. $r = - 0.6866$, $p < 0.02$.

Table I. General data on studied patients with chronic renal failure receiving regular hemodialysis treatment. Values are Mean ± SD

	Age (years)	Hd (months)*	Body weight kg	Blood urea mmol/l	Creatinin µmol/l	Serum protein g/l	Serum transferin	Heamtocrit
I with polycystic kidneys (n=8)	52.5 ± 6.1	55.7 ± 38	60.9 ± 5.9	32.8 ± 6.68	690.25 ± 39	69 ± 3.16	2.93 ± 0.4	0.38 ± 0.07
I:II t=	1.373	0.679	0.532	0.563	0.051	0.051	0.816	7.229
II with other kidney disease (n=9)	46.6 ± 9.7	70.3 ± 44	62.3 ± 4.8	33.0 ± 6.4	673.7 ± 60	68.9 ± 4.9	2.64 ± 0.85	0.19 ± 0.03

* Period of chronic hemodialysis treatment (months)

Table II. Peripheral blood values and erythropoietin level in patients with renal failure maintained on chronic hemodialysis (HD). Group I with polycystic kidney disease (RP) and Group II with other kidney diseases: pyelonephritis (PN) and glomerulonephritis (GN).

Group I	Sex/age	Diagnosis	HD months	Hb g/dl	RBC x 10^{-12}/l	Hematocrit	WBC x10^{-9}/l	Transfusion/ year	Erythropoietin bio U/ml	Erythropoietin RI mU/ml
1.	M/48	RP	59	13.3	4.80	0.42	5.8	0	0.21	240.0
2.	M/63	RP	67	12.0	4.00	0.39	5.2	2	n.a.	500.0
3.	F/57	RP	7	9.6	3.00	0.30	3.3	0	n.d.	24.2
4.	M/46	RP	107	9.8	3.00	0.31	4.3	0	0.37	158.0
5.	M/53	RP	94	9.8	3.10	0.31	5.2	0	n.d.	117.7
6.	M/56	RP	88	15.7	4.83	0.49	4.5	0	n.d.	24.1
7.	M/54	RP	20	14.6	4.30	0.44	4.3	0	0.36[1]	121.6
8.	F/43	RP	4	10.7	3.30	0.34	7.7	0	0.70[1]	87.5
Mean ± SD	52.5 ± 6.1		55.7 ± 38	11.93 ± 0.22	3.72 ± 0.66	0.38 ± 0.07	5.03 ± 1.31			159.1 ± 154
I:II t =	1.373		0.679	6.768	6.981	7.229	1.255			2.367
p value			< 0.001	< 0.001	< 0.001					< 0.05

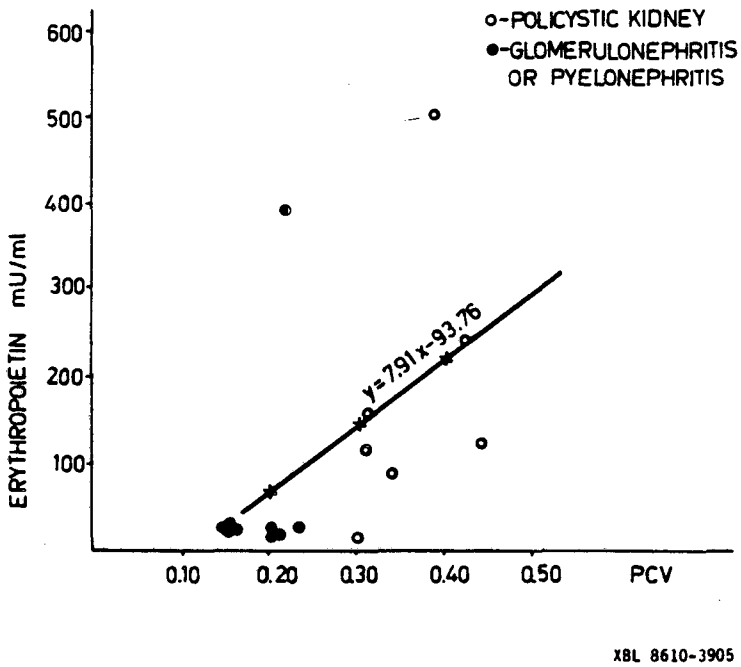
Table II (continued)

Group I	Sex/age	Diagnosis	HD months	Hb g/dl	RBC x 10^{-12}/l	Hematocrit	WBC x10^{-9}/l	Transfusion/ year	Erythropoietin bio U/ml	Erythropoietin RI mU/ml
9.	F/36	PN	81	6.4	2.00	0.20	4.4	4	n.d.	15.2
10.	F/53	PN	56	7.3	2.22	0.21	6.0	1	n.d.	16.6
11.	F/37	PN	162	7.0	2.20	0.22	7.0	3	n.d.	391.0**
12.	F/53	GN	105	6.4	2.00	0.20	4.5	7	n.a.	24.7
13.	F/48	GN	72	7.2	2.27	0.23	6.9	0	n.a.	20.8
14.	F/48	GN*	91	4.5	1.40	0.15	4.0	2	n.a.	20.9
15.	F/50	GN	12	4.8	1.50	0.15	7.3	4	n.a.	21.6
16.	F/64	PN	35	4.8	1.50	0.15	7.0	3	n.a.	28.3
17.	F/31	GN	19	5.5	1.68	0.16	5.4	5	n.a.	17.5
Mean ± SD	46.6 ± 9.7		70.3 > 44	5.98 ± 1.04	1.86 ± 0.32	0.19 ± 0.03	5.83 ± 1.29			20.7 ± 4

bio - bioassayed
* - anephric
** - not taken into calculation
n.d. - not detected
n.a. - not assayed
[1] - samples for bioassay obtained 22 months later

Table III. Inhibition of mouse bone marrow CFU-E derived colonies in methylcellulose cultures with 0.25 μ Ep and 5 or 10 percent sera of patients with chronic renal insufficiency added at the beginning of incubation. Number of colonies per 10^5 nucleated mouse bone marrow cells without addition of those sera was 606 ± 46 (4 plates per group) in this experiment. The results are presented as percentage of the control number of colonies expressed as 100%.

	Percent colonies patients sera added		Hematocrit
	5%	10%	
Group I			
1.	44	41	0.42
2.	--	--	0.39
3.	52	41	0.30
4.	56	24	0.31
5.	50	23	0.31
6.	25	--	0.49
7.	38	34	0.44
8.	44	43	0.34
Group II			
9.	53	35	0.20
10.	42	24	0.21
11.	13	10	0.22
12.	20	5	0.20
13.	54	45	0.23
14.	--	--	0.15
15.	82	38	0.15
16.	31	24	0.15
17.	100	100	0.16
Controls			
a.	94	92	
b.	97	94	
c.	100	100	


o-POLICYSTIC KIDNEY
●-GLOMERULONEPHRITIS
OR PYELONEPHRITIS
ERYTHROPOIETIN mU/ml
600
500
400
300
200
100
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0.10
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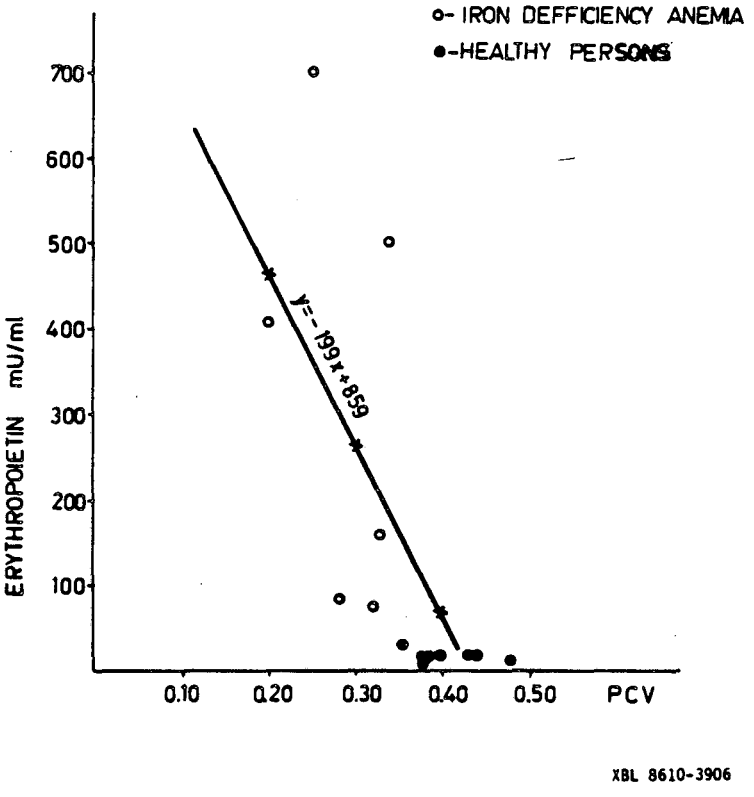
0.30
0.40
0.50
PCV
XBL 8610-3905

Figure 1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●-HEALTHY PERSONS
ERYTHROPOIETIN mU/ml
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600
500
400
300
200
100
y=-199x+859
0.10
0.20
0.30
0.40
0.50
PCV
XBL 8610-3906

Figure 2

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