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Relationship Between Renal Anemia and Prognostic Stages of IgA Nephropathy

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In 2002, the Joint Committee of the Special Study Group on Progressive Glomerular Diseases, Ministry of Health, Labor and Welfare of Japan newly revised the clinical guidelines for IgA nephropathy (Sakai et al.: *Jpn J Nephrol* 37:417–421, 1995; Tomino and Sakai: *Clin Exp Nephrol*, 7, 93–97, 2003). The prognostic stages were classified into four groups: the good prognosis group (Group I), relatively good prognosis group (Group II), relatively poor prognosis group (Group III), and poor prognosis group (Group IV). The relationship between the levels of Hb, Ht, and RBC in peripheral blood and the renal prognostic stages was determined in 62 patients with IgA nephropathy in the present study. The mean levels of Hb, Ht, and RBC were significantly lower in Group IV than in Group I ($P<0.05$). However, there were no significant changes in the levels of serum creatinine (s-Cr) or creatinine clearance (CCr) among these four groups. It appears that the levels of Hb, Ht, and RBC in peripheral blood may be important clinical parameters for the evaluation of prognostic stages in patients with IgA nephropathy. J. Clin. Lab. Anal. 19:80–83, 2005.

Key words: anemia; interstitial injury; IgA nephropathy

INTRODUCTION

In 1995 and 2002, the Joint Committee of the Special Study Group on Progressive Glomerular Diseases, Ministry of Health, Labor and Welfare of Japan, described four prognostic stages of IgA nephropathy: the good prognosis group (Group I), relatively good prognosis group (Group II), relatively poor prognosis group (Group III), and poor prognosis group (Group IV) (1,2). Fibrosis and/or inflammatory cell infiltration in the tubulointerstitial regions were marked in Group IV. The authors reported the relationship between the distribution of mast cells in the tubulointerstitium and prognosis of patients with IgA nephropathy (3). There was a significant correlation between the number of mast cells per unit area of the whole tubulointerstitium and the degree of tubulointerstitial fibrosis (3). It was recently shown that fibroblast-like interstitial cells present between the peritubular capillaries and tubular cells are the source of circulating erythropoietin (EPO) (4,5). The production of EPO, which is dependent on low oxygen pressure, may be attenuated in association with the decreased number of the corresponding fibroblastic cells and the phenotypic change to myofibroblasts in experimental renal fibrosis (6,7). These findings suggest a significant correlation between serum EPO levels and the degree of tubulointerstitial injuries. Thus, the expansion of interstitial fibrosis and/or tubulointerstitial damage may be correlated with renal anemia, probably by a reduced number of producing EPO cells. The objective of the present study was to determine the relationship between the grade of renal anemia and the renal prognostic stages in patients with IgA nephropathy.

MATERIALS AND METHODS

Patients

Biopsy specimens from patients with IgA nephropathy stained predominantly for IgA in the glomerular mesangial areas. Patients with systemic lupus erythematosus (SLE), Henoch-Schoenlein purpura (HSP) nephri-

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tis, liver cirrhosis, or other systemic diseases were excluded from this study. HSP nephritis patients who had purpura, arthralgia, and/or abdominal symptoms (pain, melena, and/or diarrhea) were also excluded. Of the 62 patients with IgA nephropathy, 25 were males and 37 were females. The ages of the patients ranged from 17 to 54 years (mean = 30.5 years). Thirteen patients with minimal change nephrotic syndrome (MCNS) were used as controls. The ages of these patients ranged from 14 to 69 years (mean = 32.2 years). None of the patients were treated with antiplatelet drugs, antiinflammatory drugs, corticosteroids, antihypertensive drugs, or immunosuppressants at the time the renal biopsy was performed.

Blood samples were obtained from all of the patients prior to renal biopsy. None of these patients showed a positive reaction for stool occult blood or a bleeding tendency. None of the female patients showed menstruation at that time.

Prognostic Criteria for Patients With IgA Nephropathy

IgA nephropathy patients were divided into four groups at the time of renal biopsy (1,2). In the good prognosis group (Group I), slight mesangial proliferation and increased matrix were observed. No glomerulosclerosis, crescent formation, or adhesion to Bowman's capsules was observed. No prominent changes were seen in the interstitium, renal tubuli, or blood vessels. In the relatively good prognosis group (Group II), slight mesangial proliferation and increased matrix were observed. Glomerulosclerosis, crescent formation, and adhesion to Bowman's capsules were observed in less than 10% of all biopsied glomeruli. Interstitial and vascular findings were the same as in Group I. In the relatively poor prognosis group (Group III), moderate, diffuse mesangial cell proliferation and increased matrix were observed. Glomerulosclerosis, crescent formation, or adhesion to Bowman's capsules were seen in 10–30% of all biopsied glomeruli. Cellular infiltration was slight in the interstitium except around some sclerosed glomeruli. Tubular atrophy was slight, and mild vascular sclerosis was observed. In the poor prognosis group (Group IV), severe, diffuse mesangial cell proliferation and increased matrix were observed. Glomerulosclerosis, crescent formation, and adhesion to Bowman's capsules were seen in more than 30% of all biopsied glomeruli. When sites of sclerosis are totaled and converted to global sclerosis, the sclerosis rate was more than 50% of all glomeruli. Some glomeruli also showed compensatory hypertrophy. The sclerosis rate was the most important index in the evaluation of prognosis. In addition to fibrosis, interstitial cellular infiltration and tubular atrophy were observed. Hyperplasia or degeneration may be present in some intrarenal arteriolar walls.

Clinical Activity

Levels of serum creatinine (s-Cr) and creatinine clearance (CCr) were measured in our hospital. The levels of hemoglobin (Hb), hematocrit (Ht), red blood cells (RBC), reticulocytes, Fe, TIBC and ferritin in peripheral blood were also measured by routine methods in our hospital. We measured EPO levels by a two-antibody radioimmunoassay using ^{125}I-labeled EPO and standard recombinant EPO samples with a commercial kit (Recombigen EPO kit; Japan DPC Co., Tokyo, Japan) (8). Normal levels of serum EPO ranged from 8 to 36 mU/mL (8). Twenty-six patients with IgA nephropathy (Group I: five patients; Group II: seven patients; Group III: nine patients; and Group IV: five patients) were used for measurement of EPO.

Statistical Analysis

Statistical analysis was performed with STAT View (Version 5.0) (9). The results are expressed as means ± SE. Student's *t*-test was also used in statistical comparisons between individual study groups. $P < 0.05$ was regarded as significant.

RESULTS

There were no significant changes in the levels of s-Cr among the four groups of patients with IgA nephropathy. There were also no significant changes in the levels of CCr among the four groups (Table 1). The mean levels of Hb, Ht, and RBC in patients with IgA nephropathy and MCNS are shown in Table 2. The mean levels of Hb, Ht, and RBC were lower in patients with IgA nephropathy than in patients with MCNS, but there was no statistical significance. The mean levels of Hb, Ht, and RBC gradually decreased according to the grading of patients with IgA nephropathy. The mean

TABLE 1. Mean level of serum Cr and CCr in patients with IgA nephropathy and MCNS*

Disease	s-Cr (mg/dl)	CCr mg/dl
IgA nephropathy (n = 62)	0.73 ± 0.02	97.998 ± 2.271
Group I (n = 12)	0.77 ± 0.03	98.89 ± 6.121
Group II (n = 18)	0.70 ± 0.03	102.99 ± 4.363
Group III (n = 19)	0.74 ± 0.03	96.38 ± 3.822
Group IV (n = 13)	0.72 ± 0.03	92.63 ± 4.275
MCNS (n = 13)	0.81 ± 0.03	106.40 ± 6.704

*Mean ± SE
s-Cr, serum creatinine; CCr, creatinine clearance.

TABLE 2. Mean level of Hb, Ht and RBC in peripheral blood and serum EPO in patients with IgA nephropathy and MCNS

Disease	Hb (mg/dl)	Ht (%)	RBC (10^{10}/I)	EPO (IU/ml)
IgA nephropathy (n = 62)	13.6 ± 0.2	40.0 ± 0.55	439.4 ± 8.8	17.39 ± 1.59 (n = 26)
Group I (n = 12)	14.3 ± 0.3	42.0 ± 0.89	471.0 ± 8.7	16.74 ± 0.84 (n = 5)
Group II (n = 18)	13.6 ± 0.3	40.0 ± 0.84	445.4 ± 8.9	16.56 ± 4.20 (n = 7)
Group III (n = 19)	13.5 ± 0.3	40.0 ± 1.05	441.6 ± 10.4	16.31 ± 2.48 (n = 9)
Group IV (n = 13)	12.9 ± 0.5[a]	37.0 ± 1.43[b]	399.1 ± 34.0[a]	21.16 ± 4.18 (n = 5)
MCNS (n = 13)	15.2 ± 0.05	46.0 ± 1.28	505.0 ± 12.0	–

*Mean ± SE.
MCNS, minimal change nephrotic syndrome.
[a] $P < 0.01$ vs. group I.
[b] $P < 0.05$ vs. group I.

level of Hb declined from 14.3 ± 0.3 mg/dl (Group I) to 12.9 ± 0.5 mg/dl (Group IV). The mean level of Hb was significantly lower in Group IV than in Group I ($P < 0.01$). However, there were no significant changes in the levels of Hb between Groups I and II, Groups II and III, or Groups III and IV (Table 2). The mean Ht level declined from 42.0% ± 0.9% (Group I) to 37.0% ± 1.4% (Group IV). The mean level of Ht was significantly lower in Group IV than in Group I ($P < 0.05$) (Table 2). However, there were no significant changes in the levels of Ht between Groups I and II, Groups II and III, or Groups III and IV. The mean RBC level declined from 471.0 ± 8.7 10^{10}/L (Group I) to 399.1 ± 34.0 10^{10}/L (Group IV). The mean level of RBC was significantly lower in Group IV than in Group I ($P < 0.01$) (Table 2). However, there were no significant changes in the levels of RBC between Groups I and II, Groups II and III, or Groups III and IV. There were significant differences in the levels of Hb, Ht, and RBC between patients with IgA nephropathy and controls with MCNS ($P < 0.05$).

The mean level of serum EPO in patients with IgA nephropathy was 17.39 ± 1.59 mU/mL (normal range = 8–36 mU/mL). The mean levels of serum EPO were 16.74 ± 0.84 mU/mL in Group I, 16.56 ± 4.20 mU/mL in Group II, 16.31 ± 2.48 mU/mL in Group III, and 21.16 ± 4.18 mU/mL in Group IV. The mean EPO level was slightly higher in Group IV than in the other groups. However, there were no significant changes in the levels among the four groups. The serum levels of Fe, TIBC, ferritin, and reticulocytes in all of the patients were within normal ranges.

DISCUSSION

In this study, the levels of Hb, Ht, and RBC gradually decreased according to progression of renal injuries in patients with IgA nephropathy. The mean levels of Hb, Ht, and RBC in peripheral blood were significantly lower in Group IV (poor prognosis group) than in Group I (good prognosis group). None of these patients showed positive reactions for stool occult blood, bleeding tendency, or menstruation in this study. EPO is a glycoprotein hormone that is produced in peritubular interstitial cells. It regulates the rate of proliferation and differentiation of erythroid precursors in the bone marrow (10). The beneficial effects of recombinant human EPO have been established for the treatment of renal anemia in chronic renal failure (11). Inomata et al. (12) reported that low serum EPO may be a new predictive marker of the progression of diabetic nephropathy. Nielsen and Thaysen (13) and Thaysen et al. (14) demonstrated EPO deficiency in patients with acute renal failure including crescentic glomerulonephritis. However, these reports did not describe the relationship between serum EPO levels and renal tubular damage. Recent molecular studies have shown that EPO production that is responsive to hypoxia is mediated by hypoxia-inducible factor-1 (HIF-1), which also stimulates platelet-derived growth factor (PDGF) and vascular endothelial growth factor (15,16). It is generally considered that various inflammatory cytokines such as tumor necrosis factor IL-1 and transforming growth factor β(TGF β) may induce glomerular injuries in several types of renal diseases, including IgA nephropathy. PDGF induces mesangial cell proliferation, and TGF β contributes to the fibrogenic process (17). These factors are known to be antagonists of EPO production (18,19). Toxic metabolites produced in renal failure are also considered to be antagonisits of EPO synthesis (11). Maxwell et al. (7) proposed that the proportion of EPO-producing cells may be decreased among interstitial fibroblast-like cells bearing the myofibroblast phenotype in experimental models of interstitial renal disease. These cells are partially refractory to hypoxic stimulation of EPO production. Fibrosis and/or inflammatory cell infiltration in the tubulointerstitial region were marked in Group IV among the four groups in this study. In 1999, Machiguchi et al. (20) reported that the mean level of

serum EPO in patients with IgA nephropathy (17.7 ± 6.3 mU/mL) did not differ from the control level (19.3 ± 3.7 mU/mL). They indicated that serum EPO levels that show an inverse correlation with s-Cr, blood pressure levels, and heavy proteinuria may reflect the clinical severity of IgA nephropathy (20). Thus, it is postulated that EPO production in Group IV may be decreased compared to that in other groups. However, the mean levels of serum EPO were slightly higher in Group IV than in other groups in this study, although there were no significant changes in these levels among the four groups. It is difficult to clarify the increase of serum EPO levels in Group IV. Machiguchi et al. (20) showed that the mean s-Cr level was 0.95 ± 0.43 (range = 0.5–2.2 mg/dl). In this study, there were no significant changes in the levels of s-Cr and CCr among the four groups of IgA nephropathy. The levels of s-Cr and CCr in Group IV showed normal ranges. These findings suggest that hyperproduction of EPO may occur in the resident peritubular interstitial cells. On the other hand, the circadian rhythm of serum EPO levels indicated that the basal level of serum EPO occurred in the morning and the peak level (a 60% increase) occurred at 8:00 PM (21). Since the time of blood sampling differed for each patient in this study, it will be necessary to measure the levels of EPO at 8:00 PM in many patients with IgA nephropathy in future studies. The serum levels of Fe, ferritin, TIBC, and reticulocytes in the peripheral blood of all patients did not decrease in this study. There were no significant differences in the levels of Hb, Ht, and RBC between Group II (relatively good prognosis group) and Group III (relatively poor prognosis group). However, the levels of Hb, Ht, and RBC were markedly decreased in Group IV. It appears that levels of Hb, Ht, and RBC in peripheral blood may be important clinical parameters for evaluating a prognosis prior to renal biopsy.

REFERENCES

1. Sakai H, Abe K, Kobayashi Y, et al. Clinical guidelines of IgA nephropathy. Jpn J Nephrol 1995;37:417–421.
2. Tomino Y, Sakai H. Clinical guidelines of IgA nephropathy in Japan, 2nd version. Clin Exp Nephrol 2003;7:93–97.
3. Kurusu A, Suzuki Y, Horikoshi S, Shirato I, Tomino Y. Relationship between mast cells in the tubulointerstitum and prognosis of patients with IgA nephropathy. Nephron 2001;89:391–397.
4. Lacombe C, Da Silva JL, Bruneval P, et al. Peritubular cells are the site of erythropoietin synthesis in the murine hypoxic kidney. J Clin Invest 1988;81:620–623.
5. Maxwell PH, Osmond MK, Pugh CW, et al. Identification of the renal erythropoietin-producing cells using transgenic mice. Kidney Int 1993;44:1149–1162.
6. Tan CC, Tan LH, Eckardt KU. Erythropoietin production in rats with post-ischemic acute renal failure. Kidney Int 1996;50: 1958–1964.
7. Maxwell PH, Ferguson DJ, Nicholls LG, Johnson MH, Ratcliffe PJ. The interstitial response to renal injury: fibroblast-like cells show phenotypic changes and have reduced potential for erythropoietin gene expression. Kidney Int 1997;52:715–724.
8. Kaneo K, Matsui K, Moriuma H, et al. Fundamental evaluation of the radioimmunoassay kit for erythropoietin (EPO) [in Japanese]. Igakuto Yakugaku 1989;21:659–664.
9. Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. Clin Chem 1993;39:561–577.
10. Spivac J. The mechanism of action of erythropoietin. Int J Cell Cloning 1986;4;139–166.
11. Erslev AJ, Besarab A. Erythropoietin in the pathogenesis and treatment of the anemia of chronic renal failure. Kidney Int 1997;51:622–630.
12. Inomata S, Itoh M, Imai H, Sato T. Serum levels of erythropoietin as a novel marker reflecting the severity of diabetic nephropathy. Nephron 1997;75:426–430.
13. Nielsen OJ, Thaysen JH. Erythropoietin deficiency in acute tubular necrosis. J Intern Med 1990;227:373–380.
14. Thaysen JH, Nielsen OJ, Brandi L, Szpirt W. Erythropoietin deficiency in acute crescentic glomerulonephritis and in total bilateral renal cortical necrosis. J Intern Med 1991;229:363–369.
15. Semenza GL, Wang GL. A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. Mol Cell Biol 1992;12:5447–5454.
16. Wang GL, Smenzaa GL. General involvement of hypoxia inducible factor 1 in transcriptional response to hypoxia. Proc Natl Acad Sci U S A 1993;90:4304–4308.
17. Faquin WC, Schneider TJ, Goldberg MA. Effect of inflammatory cytokines on hypoxia-induced erythropoietin production. Blood 1992;79:1987–1994.
18. Jelkmann W, Pagel H, Wolff M, Fandrey J. Monokines inhibiting erythropoietin production in human hepatoma cultures and in isolated perfused rat kidneys. Life Sci 1991;50:301–308.
19. Schena FP, Gesualdo L, Grandaliano G, Montinard V. Progression of renal damage in human glomerulonephritis: is there sleight of hand in winning the game? Kidney Int 1997;52: 1439–1457.
20. Machiguchi T, Yoshida H, Yonemoto S, et al. Does circulating erythropoietin reflect progression of IgA nephropathy? Comparison with urinary N-acetyl-β-D-glucosaminidase. Nephrol Dial Transplant 1999;14:635–640.
21. Wide L, Bengtsson C, Birgegard G. Circadian rhythm of erythropoietin in human serum. Br J Hematol 1989;72:85–90.