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Serum Levels of Vascular Endothelial Growth Factor and Disease Activity in Patients with IgA Nephropathy

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To investigate if serum levels of vascular endothelial growth factor (VEGF) reflect disease activity of IgA nephropathy, the serum VEGF levels were measured in 31 patients with IgA nephropathy and 13 patients with focal glomerulosclerosis (FGS) without glomerular IgA deposition as controls using a sandwich ELISA method. The serum VEGF levels of patients with advanced stage IgA nephropathy (70.59 ± 7.84 pg/ml) were significantly higher than those in mild stage IgA nephropathy (37.36 ± 3.8 pg/ml), FGS (34.14 ± 4.87 pg/ml) and the healthy controls (22.6 ± 3.8 pg/ml). The results demonstrated that advanced stage IgA nephropathy patients with heavy proteinuria and the presence of glomerular crescents had high serum VEGF levels. This suggested that measurement of serum VEGF levels is potentially useful in evaluating the degree of renal injury in patients with IgA nephropathy.

Introduction

IgA nephropathy is well recognized as one of the most common primary glomerulonephritides and is characterized by mesangial deposition of IgA in renal specimens[1]. Some factors are known to be associated with the initiation and progression of IgA nephropathy[2]. Several investigators have shown that macrophages play an important role in the pathogenesis of mesangial hypercellularity, and irreversible glomerular damage and interstitial tissue injury in IgA nephropathy[3)~5)]. Macrophage proliferation has recently been reported to be a feature of the aggressive forms of human glomerulonephritis[6], and the degree of local macrophage proliferation may be a useful prognostic indicator for human glomerulonephritides, including IgA nephropathy[7]. There has been much interest in the mechanism by which macrophages accumulate within the kidney during disease. The entry of blood monocytes into the injured kidney is regulated by a variety of chemokines[8)9)], however, the precise mediators which signal lymphocytes and monocytes to migrate and colonize the kidney are still unclear.

Vascular endothelial growth factor (VEGF) is a specific growth factor that differs from the majority of other growth factors, which stimulate proliferation of several types of cells[10]. VEGF is thought to be an important factor regulating angiogenesis during fetal development, wound healing, and the growth of benign and malignant tumors[11]. In addition to its angiogenic properties, VEGF dilates arteries, increases capillary permeability[12] and collagenase activity[13], and exerts a

chemotactic action on monocytes[14]. VEGF also appears to participate in inflammatory processes by inducing plasma extravasation and endothelial proliferation[14)15)]. We recently reported that the serum VEGF concentrations of patients with rapidly progressive glomerulonephritis are significantly higher than in healthy volunteers and that there is no correlation between the serum levels of VEGF and serum creatinine or proteinuria[16].

The purpose of this study was to determine if high serum VEGF levels reflect the renal injury in IgA nephropathy.

Subjects and Methods

Subjects

Thirty-one patients with primary IgA nephropathy and 13 patients with focal glomerulosclerosis (FGS) without glomerular IgA deposition were examined. IgA nephropathy patients whose biopsy specimens stained predominantly for IgA in the glomerular mesangial areas were included in this study, after excluding patients with lupus nephritis, Henoch-Schönlein purpura nephritis, liver cirrhosis, and other systemic diseases. Twenty-two age-matched healthy adults were enrolled as controls. All patients gave their informed consent to participate in the study.

The histopathological changes of IgA nephropathy were divided into two stages according to the classification described previously[17]. In brief, the mild stage (n=16) was characterized by minimal or slight mesangial thickening with an increase in the homogeneous PAS-positive mesangial matrix, mild mesangial cell proliferation was observed. The advanced stage (n=15) was characterized by diffuse mesangial thickening, mesangial cell proliferation with or without capsular adhesion, fibrocellular crescents, and/or glomerular sclerosis. Glomerular capillary walls were thickened by extension of the mesangial matrix. More widespread interstitial cell infiltration and fibrosis, and tubular atrophy were also observed.

Measurement of Serum VEGF

As previously described[16], serum VEGF levels were measured by sandwich ELISA using a commercial ELISA kit (Immuno Biological Laboratories, Fujioka, Gunma, Japan) with a slight modification. In brief, serum samples or standards ($VEGF_{165}$) were incubated in wells coated with anti-human $VEGF_{165}$ monoclonal antibody. After washing, a peroxidase-conjugated anti-human $VEGF_{121}$ monoclonal antibody was added to the microwells and incubated. After another washing, the peroxidase substrate was mixed with chromogen and allowed to perform an additional incubation. An acid solution was then added to each well to terminate the enzyme reaction and to stabilize the developed color. The optical density of each well was measured at 492 nm with a microplate reader (Dynatech, Chantilly, Virginia, USA). The serum VEGF concentration was calibrated from a dose-response curve based on VEGF standards (R and D Systems, Minneapolis, Minn, USA). The sensitivity and specificity of the serum VEGF determinations were assessed as previously described[18].

Parameters of Disease Activity

The following clinical parameters at the time of renal biopsy were used to assess the disease activity: proteinuria (g/day), hematuria (/HPF), creatinine clearance, and the presence of glomerular crescents. Based on these parameters, the sensitivity and specificity of serum VEGF for detecting disease activity of IgA nephropathy in the present study were 100 and 30%, respectively.

Statistical Analysis

Student's *t* test, Mann-Whitney *U* test, and regression analysis were used for statistical analysis of the clinical data. Values are expressed as means ± SE, and $p<0.05$ was regarded as significant.

Table 1 Serum VEGF levels and laboratory data in patients with mild-stage IgA nephropathy

Patient	Age/Sex	Serum VEGF (pg/ml)	Proteinuria (g/day)	Hematuria (RBC/HPF)	Crescent	Ccr (ml/min)
1	44/F	27.1	2.4	10	−	89
2	33/M	54.3	2.3	100	−	83.8
3	26/F	22.4	2.6	30	−	76
4	51/M	53.1	1.1	8	−	89
5	30/M	29.9	0.8	20	−	117
6	23/M	48.9	0.4	50	−	110
7	55/M	62.6	1.4	10	−	129
8	19/F	30.0	0.8	10	−	75
9	53/F	34.8	1.6	20	−	64
10	22/M	22.7	0.4	20	−	89
11	38/M	26.7	0.3	30	−	50
12	46/F	25.8	1.7	numerous	−	67
13	29/M	57.2	1.5	numerous	−	90
14	33/F	28.2	1.4	50	−	82
15	21/M	55.8	2.1	numerous	−	95
16	41/M	18.6	0.2	10	−	100
Mean ± SE		37.4±3.8	1.3±0.2			87.8±5.0

VEGF : vascular endothelial growth factor, Ccr : creatinine clearance.

Results

The serum VEGF levels of the healthy controls were 22.6 ± 3.8 pg/ml. The serum VEGF levels were lower than those reported previously from our laboratory[19]. This is based on the reason why the primary antibody against $VEGF_{165}$ was changed due to the crossreactivity of previous antibody. The serum VEGF levels and laboratory data are summarized in Table 1 (mild-stage IgA nephropathy), Table 2 (advanced-stage IgA nephropathy), and Table 3 (FGS). The serum VEGF levels in the advanced stage of IgA nephropathy were significantly higher than in FGS without IgA deposition and in the healthy controls (Fig. 1), and the difference between the serum VEGF levels in the mild and advanced stage of IgA nephropathy was significant ($p<0.01$).

The urinary protein levels in advanced-stage IgA nephropathy were significantly higher than in the mild stage ($p<0.01$). The grade of microscopic hematuria in advanced-stage IgA nephropathy was significantly higher than in the mild-stage of the disease. Glomerular crescents were observed in 8 of the 15 patients with advanced-stage IgA nephropathy, but in only one patient with mild-stage IgA nephropathy (Tables 1, 2). The serum VEGF levels in both stages of IgA nephropathy with glomerular crescents 80.12 ± 38.4 pg/ml) were significantly higher than in cases without glomerular crescents (42.67 ± 20.9 pg/ml) ($p<0.01$; Fig. 2). As shown in Fig. 3, the serum VEGF levels were positively correlated with the degree of proteinuria in advanced-stage IgA nephropathy ($r=0.54$, $p<0.05$). However, there was no significant correlation between the serum VEGF levels and creatinine clearance in the patients with either stage of IgA nephropathy (data not shown).

Discussion

VEGF expression in normal glomeruli has been reported to be localized in visceral glomerular epithelial cells[20~22]. Shulman et al[23] showed that the number of VEGF-expressing cells is decreased in the glomeruli of many glomerular dis-

Table 2 Serum VEGF levels and laboratory data in patients with advanced-stage IgA nephropathy

Patient	Age/Sex	Serum VEGF (pg/ml)	Proteinuria (g/day)	Hematuria (RBC/HPF)	Crescent	Ccr (ml/min)
1	38/M	47.1	1.6	numerous	+	62
2	44/M	56.3	1.5	50	+	58
3	58/M	87.0	6.0	10	+	44
4	41/F	62.1	0.3	numerous	−	53
5	47/M	52.0	2.2	100	+	67
6	55/M	82.5	1.9	50	+	49
7	62/F	122.9	4.3	100	+	28
8	37/F	58.9	1.2	numerous	−	64
9	59/F	151.5	5.6	100	+	47
10	69/F	70.2	5.5	30	+	30
11	28/M	67.3	4.6	100	−	67
12	23/F	38.7	2.2	30	−	46
13	26/F	56.6	4.1	numerous	−	62
14	40/M	48.5	1.8	numerous	−	70
15	52/F	57.1	2.5	30	+	52
Mean ± SE		70.6 ± 7.84	3.0 ± 0.5			53.3 ± 3.3

VEGF : vascular endothelial growth factor, Ccr : creatinine clearance.

Table 3 Serum VEGF levels and laboratory data in patients with FGS

Patient	Age/Sex	Serum VEGF (pg/ml)	Proteinuria (g/day)	Hematuria (RBC/HPF)	Crescent	Ccr (ml/min)
1	19/M	29.7	0.4	10	−	110
2	50/F	15.9	2.3	20	−	65
3	25/F	30.5	0.9	−	−	98
4	42/M	47.5	1.6	−	−	69
5	33/M	21.2	1.8	−	−	90
6	34/M	30.0	3.2	10	+	48
7	66/M	21.4	7.1	5	−	72
8	56/M	44.5	4.5	−	−	117
9	19/M	29.7	0.5	−	−	114
10	39/F	33.3	2.6	10	−	51
11	39/M	10.6	2.1	−	−	91
12	21/M	53.7	3.1	10	+	45
13	23/F	75.9	1.5	3	−	66
Mean ± SE		34.1 ± 4.87	2.4 ± 0.5			79.7 ± 7.0

FGS : focal glomerulosclerosis, VEGF : vascular endothelial growth factor, Ccr : creatinine clearance.

eases. These findings suggest that damage to visceral glomerular epithelial cells in a variety of glomerular diseases may cause the release of relatively large amounts of VEGF locally, leading to increased glomerular permeability. On the other hand, marked expression of VEGF mRNA has been reported in acutely hypoxic proximal and distal tubules in both the cortex and medulla[24]. However, the function of VEGF constitutively synthesized in the visceral glomerular epithelial cells of the adult kidney is still unknown. We previously measured serum VEGF levels in

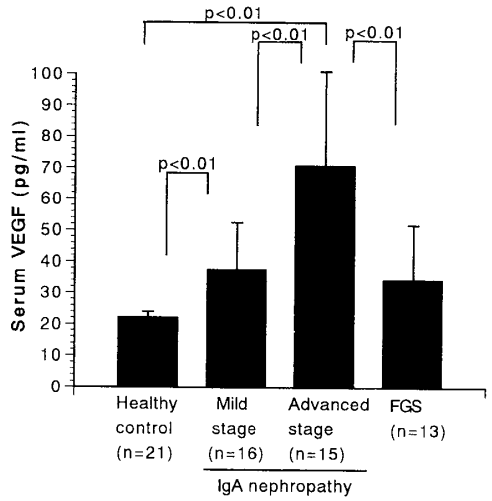

Fig. 1 Levels of serum vascular endothelial growth factor (VEGF) in IgA nephropathy, focal glomerulosclerosis (FGS), and healthy controls
The Mann-Whitney *U* test was used for statistical analysis. Values are mean ± SE.

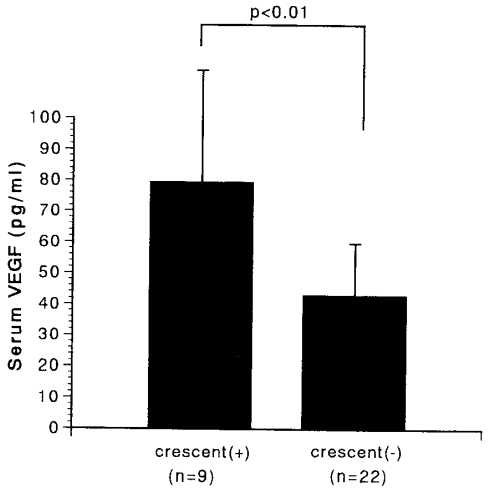

Fig. 2 Levels of serum vascular endothelial growth factor (VEGF) in IgA nephropathy patients with or without glomerular crescents
The Student's *t* test was used for statistical analysis. Values are mean ± SE.

various types of glomerulonephritis and found a significant increase in serum VEGF levels in rapidly progressive glomerulonephritis and the absence of any elevation of serum VEGF in minimal change nephrotic syndrome, IgA nephropathy, and FGS, in which crescent formation was not observed[16]. Our results suggested a possible role of

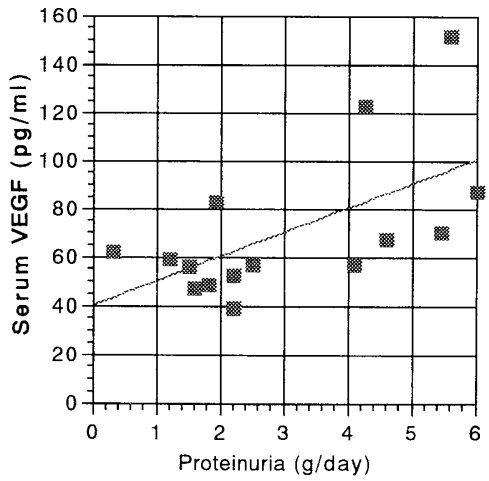

Fig. 3 Correlation between serum vascular endothelial growth factor (VEGF) levels and degree of proteinuria in advanced-stage IgA nephropathy (n=16)

VEGF in the crescent formation in rapidly progressive glomerulonephritis.

In the present study, high levels of serum VEGF were observed in patients with the advanced stage of IgA nephropathy. The serum VEGF measurements showed high sensitivity for determination of the disease activity in IgA nephropathy. The advanced stage IgA nephropathy patients with heavy proteinuria and the presence of glomerular crescents in this study had the high serum VEGF levels. There was no significant relationship between the serum VEGF levels and the kinds or amounts of drugs taken by the patients with IgA nephropathy. Since the histopathological changes in the advanced stage of IgA nephropathy were characterized by diffuse mesangial cell proliferation and tubulointerstitial injury[17], measurement of serum VEGF may be of value in estimating the degree of renal lesions in such patients. Increased serum VEGF levels were also observed in FGS, but they were lower than in advanced stage IgA nephropathy. Therefore, high serum VEGF levels are not considered specific for IgA nephropathy, but they do suggest increased disease activity in IgA nephropathy.

Macrophages are thought to be an important constituent of glomerular crescents[25], and the intensity of the macrophage infiltrate correlates with loss of renal function and histologic damage at the time of renal biopsy in IgA nephropathy[5]. We recently reported finding that increased serum VEGF levels are significantly correlated with the frequency of glomerular crescents, the grade of interstitial injury, and glomerular infiltration rate by macrophages/monocytes in human crescentic glomerulonephritis[19]. Monocytes are known to produce VEGF[26][27] and the monocyte migration in response to VEGF is mediated by the VEGF receptor, flt-1[28]. Monocytes attached to vessels during vascular rejection or vasculitis after kidney transplantation show a strong VEGF mRNA signal[24]. Therefore, monocyte-derived VEGF may be in part associated with the increased serum VEGF levels in IgA nephropathy. This is the first report describing a possible role of VEGF in the pathogenesis of IgA nephropathy. Taken together, the above findings suggest that measurement of serum VEGF is useful in estimating the degree of renal injury in IgA nephropathy.

Conclusion

We conclude that measurement of serum VEGF levels is potentially useful in evaluating the degree of renal injury in patients with IgA nephropathy.

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IgA 腎症患者における血清 vascular endothelial growth factor 濃度と疾患活動性

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当教室で腎生検によりIgA腎症と組織診断が得られ，臨床データ解析が可能であった31症例を対象とした．対照として，22例の健常者から採血した．IgA腎症は，軽症群と進行群に分けて検討した．比較症例として，IgA沈着を認めない巣状糸球体硬化症(FGS)を無作為に選択した．すべての症例はインフォームドコンセントを得たのち検討した．IgA腎症における疾患活動性を判定するために，血清vascular endothelial growth factor（VEGF）濃度の測定が有用であるか検討するのが，本研究の目的である．血清VEGF濃度は，$VEGF_{165}$と$VEGF_{121}$を認識する2種類の抗体を用いたsandwich ELISAで測定した．疾患活動性として，一日尿蛋白量，血尿の程度，クレアチニン・クリアランス(Ccr)および糸球体における半月体形成率を用いた．進行群のIgA腎症における血清VEGF濃度（70.6±7.8 pg/ml）は，健常者（22.6±3.8pg/ml），軽症群のIgA腎症（37.4±3.8pg/ml）およびFGS症例（34.1±4.9pg/ml）に比し，有意に高値を示した（$p<0.01$）．また，IgA腎症においては，半月体形成率の多い群で，血清VEGF濃度は有意に高値を示した（$p<0.01$）．血尿の程度との相関はなかったが，進行群のIgA腎症における血清VEGF濃度と尿蛋白量との間には，正の相関（$r=0.54$, $p<0.05$）が認められた．よって，IgA腎症の血清VEGF濃度は，疾患活動性を反映している可能性がある．