CHAPTER IV

RESULTS

1. Samples Characteristics

Forty-one kidney biopsy samples of lupus nephritis were studies. Histopathologic study was performed by a pathologist (V.K.) who was not aware of the results of the molecular study. Nine samples with number of glomeruli less than five were considered inadequate and discarded. Patient characteristics were summarized in table 7. Eighty-four percent (n=26) were focal or diffuse proliferative lupus nephritis (class III or class IV) and sixteen percent (n=6) were other classes. Most patients received standard immunosuppressive treatment including prednisolone and cyclophosphamide or mycophenolate mofetil. Six kidney biopsy samples from kidney transplant donors were used as controls.

Table 7. Samples Characteristics

Characteristics	LN	Kidney donor
Number	32	6
Gender (F/M)	32/0	3/3
Age (year)	29.5 (34-23)*	48.5 (55.3-36.50)*
Serum Creatinine (mg/dl)	1.0 (2.2-0.7)*	1.0 (1.1-0.7)*
Protienuria (g/day)	2.0 (3.61-1.11)*	0
Urinary erythrocyte count (per high power)	12 (40 -1)*	0
Activity score	5 (11-0.5)*	N/A
Chronicity score	2 (6-1)*	N/A

* Inter-quartile range (Q_3-Q_1)

2. Intra-Renal VEGF mRNA Levels in Lupus Nephritis

The intra-renal VEGF mRNA level was significantly decreased in patients with lupus nephritis as compare with kidney transplant donors (control) (-0.83 ± 0.7 vs. -0.001± 0.39 log copies; p=0.002) (Figure 4).

3. Intra-Renal VEGF mRNA Levels were Associated with the Severity of Renal Pathology

The high percentage (≥ 19%) of glomerular endocapilary proliferation was associated with decreased VEGF mRNA level (Figure 5A) and the presence of crescent formation was inversely associated with the VEGF levels as well (Figure 5B) (p-value = 0.01 and 0.04, respectively). The high renal activity index (≥ 2.5) was also associated with decreased VEGF mRNA levels (Figure 5C) (p-value = 0.03). There was no correlation between the other histological parameters and the VEGF mRNA levels.

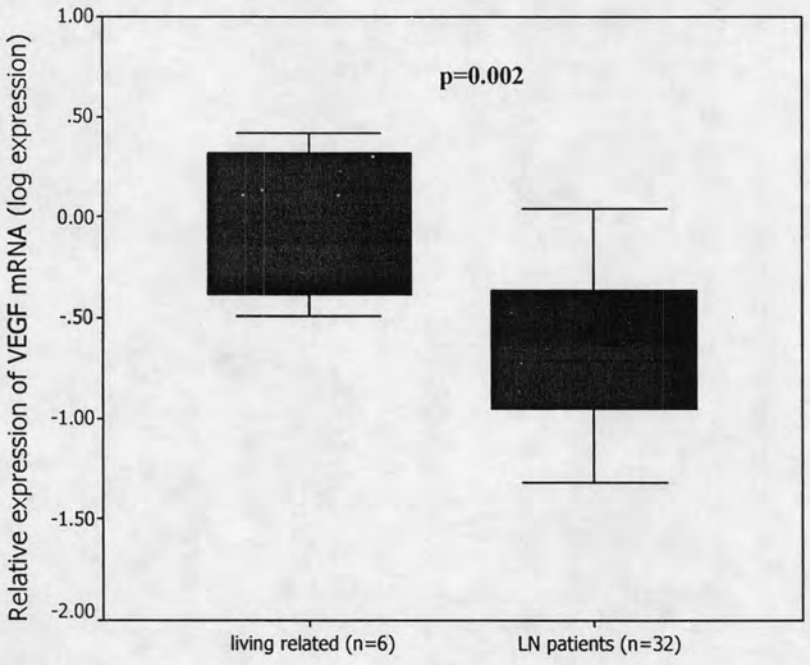
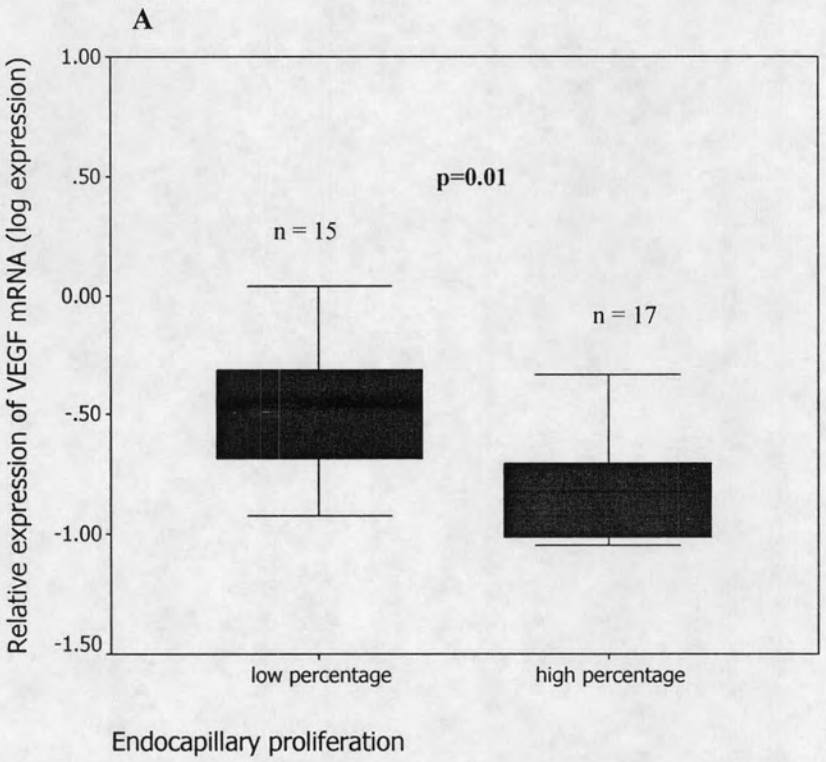
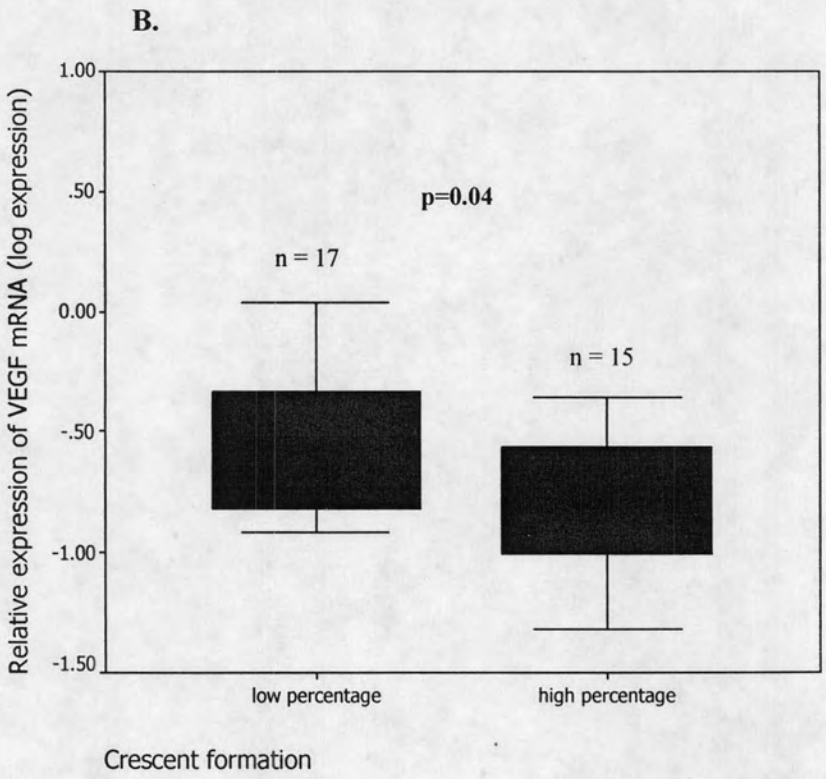


Figure 4. Levels of VEGF mRNA in kidney biopsy. Bar show (log)mRNA levels and means for VEGF in kidney biopsy tissues. The level of VEGF was significantly higher in kidney donors (n=6) than in lupus nephritis (n=32) (p=0.002).


A
1.00
.50
0.00
-.50
-1.00
-1.50
Relative expression of VEGF mRNA (log expression)
p=0.01
n = 15
n = 17
low percentage
high percentage
Endocapillary proliferation


B.
1.00
.50
0.00
-.50
-1.00
-1.50
Relative expression of VEGF mRNA (log expression)
p=0.04
n = 17
n = 15
low percentage
high percentage
Crescent formation

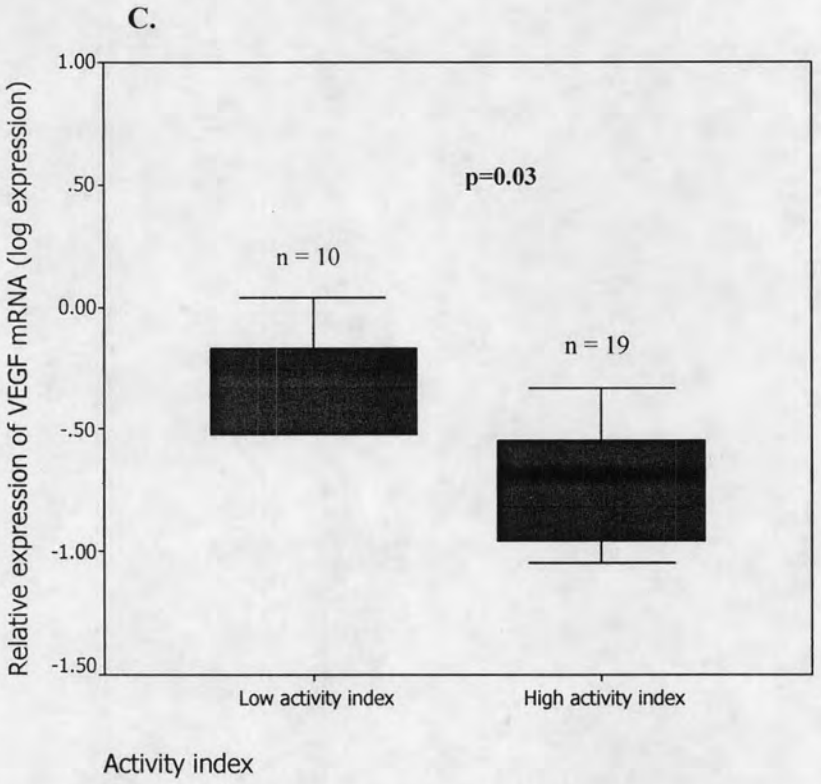


Figure 5. The boxplots of VEGF mRNA levels with three different pathological groups. Boxplots show VEGF mRNA levels among three different pathological groups. The levels of VEGF were inversely associated with the three parameters. A) glomerular endocapillary proliferation (p=0.01), B) crescent formation (p=0.04) and C) activity index (p=0.03)

4. Intra-Renal VEGF Protein Expression

The Immunostaining demonstrated VEGF protein tends to difference in lupus nephritis patients versus kidney donors. It level was toward decrease in patient who has classified in server form. In kidney donor, there was constitutive expression of VEGF protein while it in patient was tended decreasing (Figure 6). Moreover, the patient who was diagnosed as class IV LN and classified in serious pathological features (high percentage of endocapillary proliferation, crescent formation and activity index) obviously decreased VEGF protein staining in glomeruli (Figure 7 and 8). Kidney tubules

and capillaries were normally formation and expressing VEGF protein in donor while that tend to abnormal in patient (Figure 6).

5. The Association of Intra-Renal VEGF mRNA Levels and Renal Outcomes in Lupus Nephritis Patients

The pre-defined renal outcomes of lupus nephritis were a doubling of serum creatinine, and end-stage renal disease (ESRD). Seven patients (22%) developed end-stage renal disease (ESRD) and five patients (16%) had a doubling of serum creatinine during 180 days of follow up. The patients were divided into low and high levels of VEGF mRNA using the 95% confidence interval for the mean at upper bound value as the cutoff point (-0.57). The association of intra-renal VEGF gene expression and renal outcomes were showed with the cumulative survival curves that derived by the Kaplan-Meier method (Figure 9) and the differences between survivals curves were compared by the log-rank test (Table 9). Patients with low VEGF levels had significantly poor renal outcome. The patients who were classified in low VEGF gene expression group exhibited significant association with the ESRD, doubling serum creatinine or combination of both outcomes (p-value = 0.01, 0.04 and 0.005, respectively). The demographics of patient with low and high level VEGF were showed in table 8.

Moreover histological parameter, including endocapillary proliferation, crescent formation and activity index, were analyzed with renal outcomes. We found some parameter associated with poorly renal outcomes (Table 9).

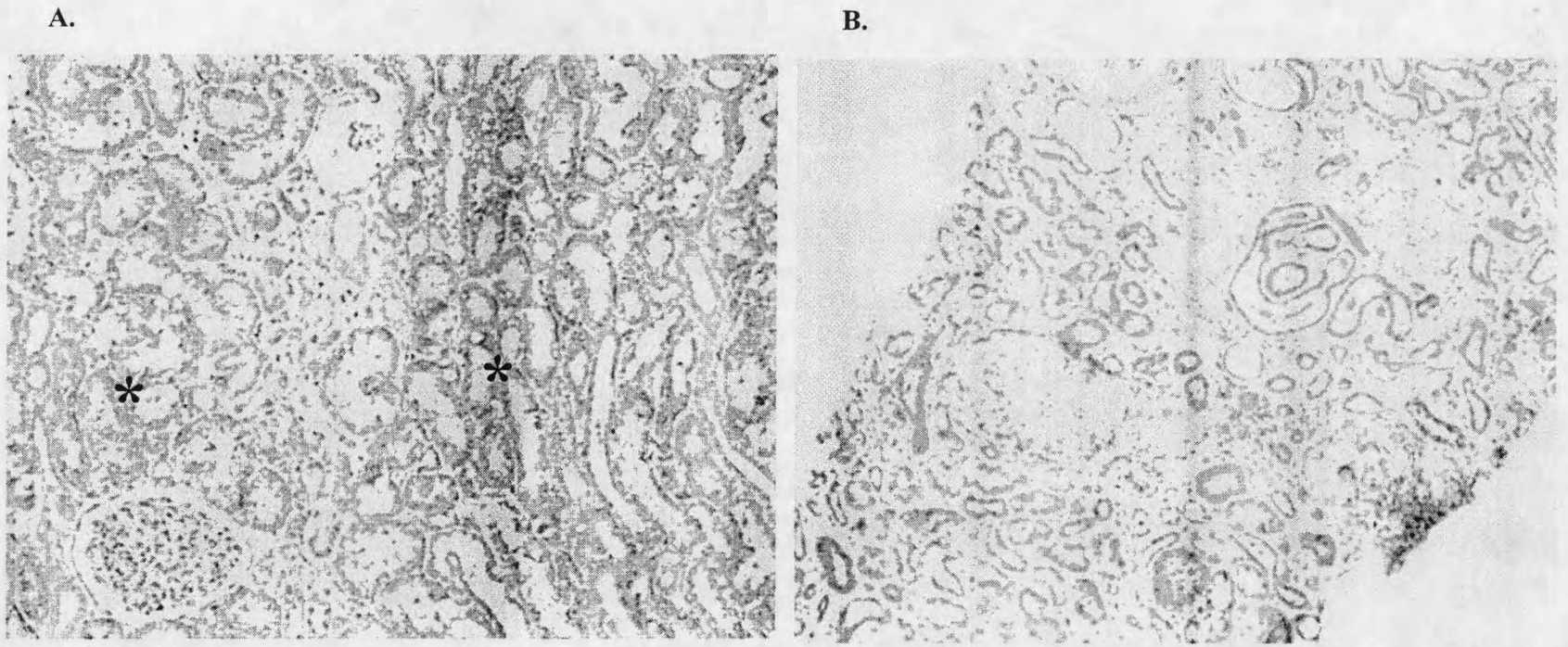


Figure 6. Intra-renal VEGF protein expression in lupus nephritis. VEGF is constitutively expressed in tubular and capillaries of kidney donors (A: asterisk), while it tends to decreasing in patient (B). Magnification: x40.

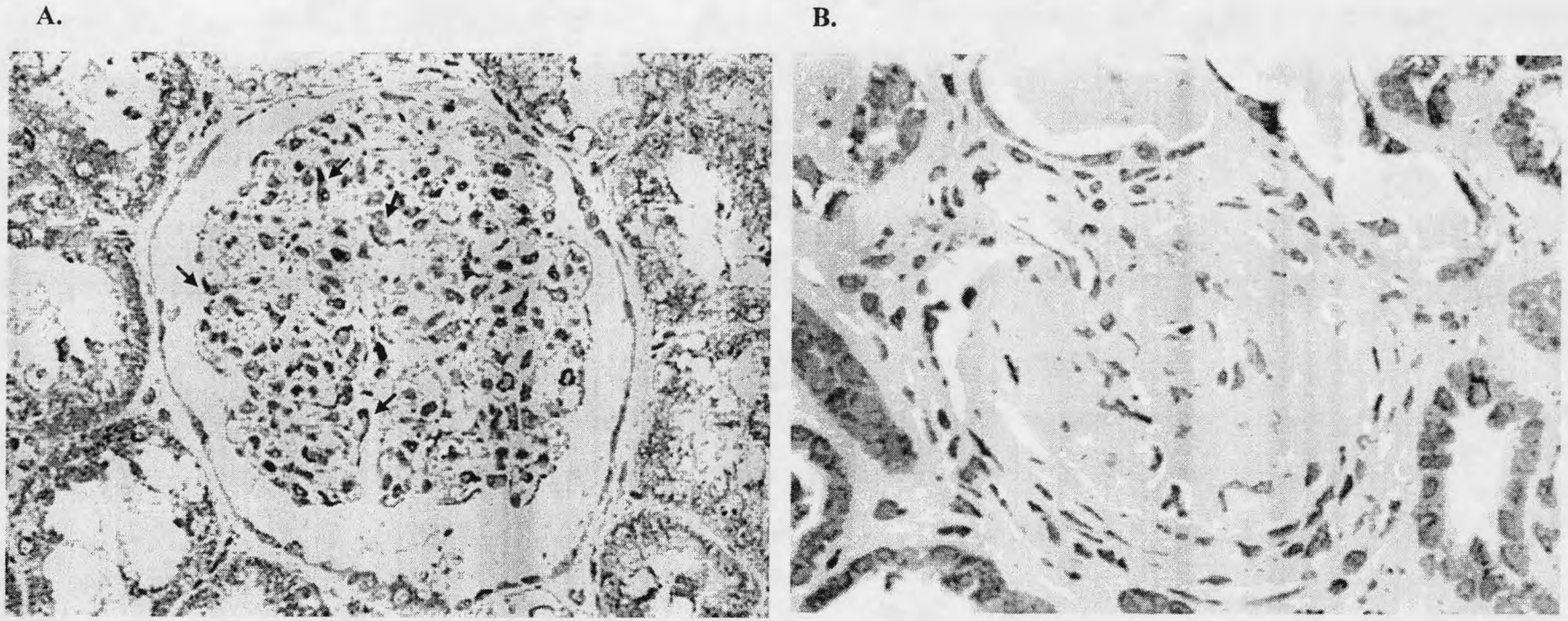


Figure 7. Glomerular VEGF protein expression. The patient who was diagnosed as class IV LN and classified in serious pathological features obviously decreased glomerular VEGF protein expression (B) whiles the donor constitutively (A). (podocytes staining, arrow) Magnification: x400.

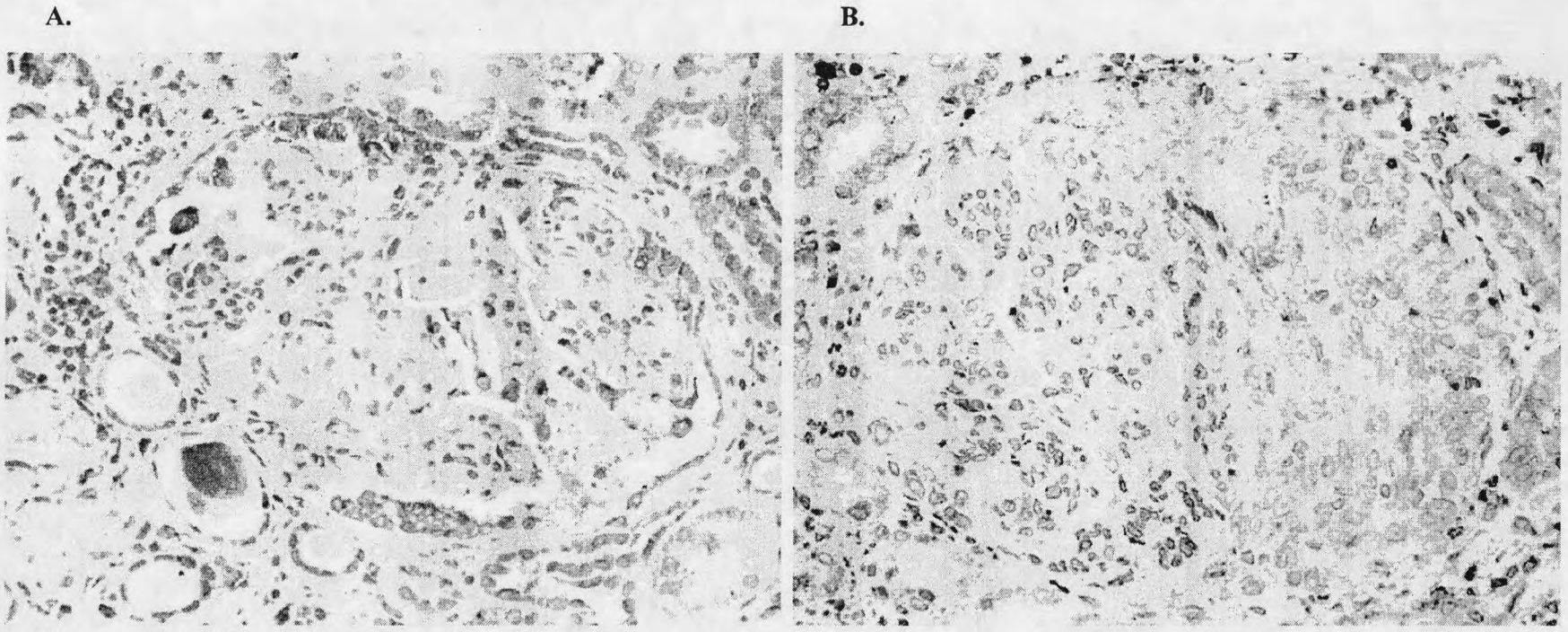
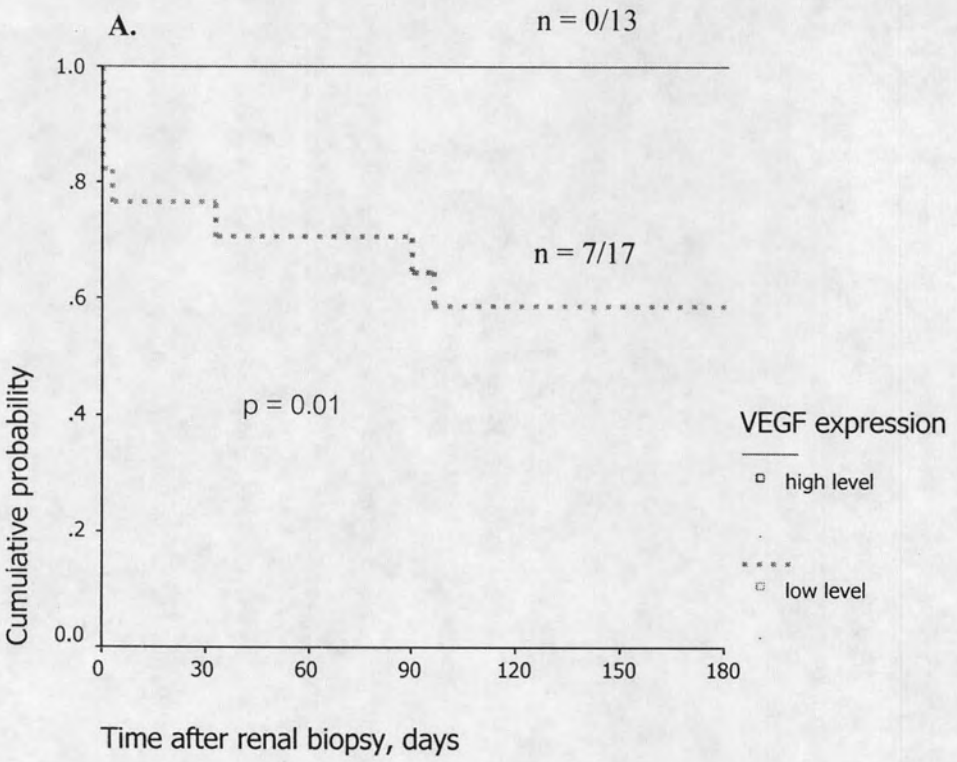
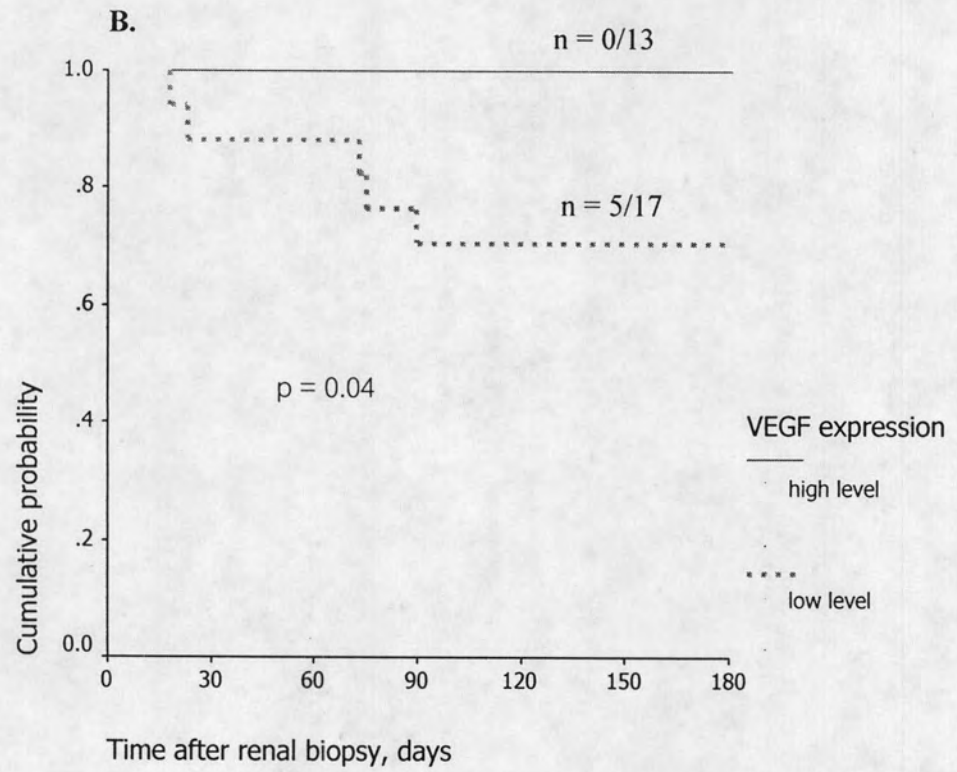


Figure 8. Glomerular VEGF protein expression in serious pathological features. The patient who was diagnosed as class IV LN and high percentage of endocapillary proliferation and crescent formation presented decreasing VEGF protein staining (A: high endocapillary proliferation, B: crescent formation). Magnification: x400.


A.
n = 0/13
n = 7/17
p = 0.01
Cumulative probability
1.0
.8
.6
.4
.2
0.0
0
30
60
90
120
150
180
Time after renal biopsy, days
VEGF expression
high level
low level


B.
n = 0/13
n = 5/17
p = 0.04
Cumulative probability
1.0
.8
.6
.4
.2
0.0
0
30
60
90
120
150
180
Time after renal biopsy, days
VEGF expression
high level
low level

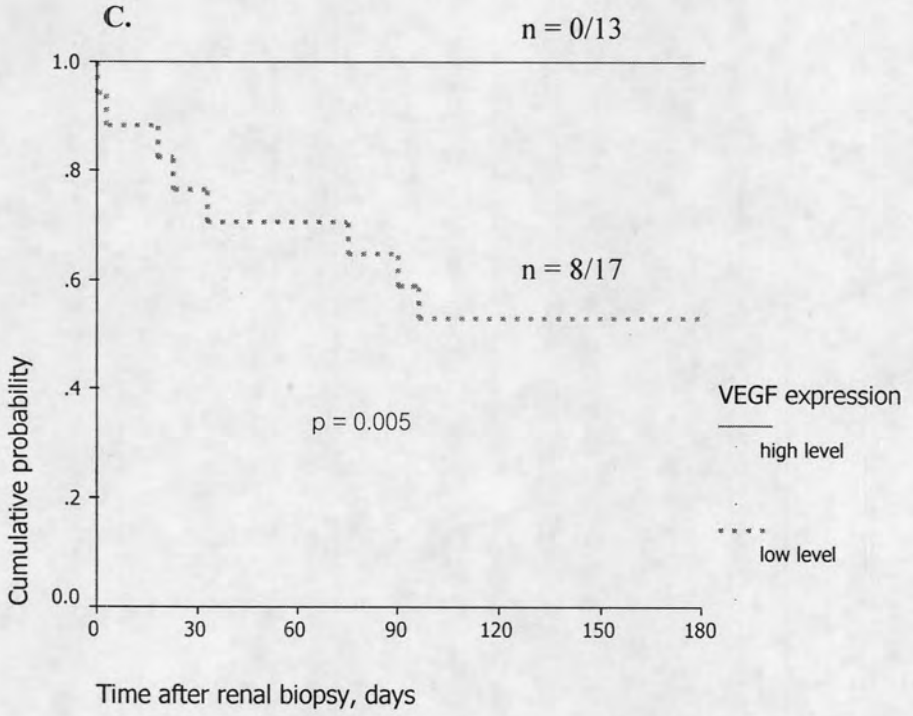


Figure 9. The cumulative probability to remain free of renal outcomes, end-stage renal disease (ESRD) (A), doubling serum creatinine (B) and combination of both outcomes (C) for lupus nephritis patients with low VEGF gene expression and high VEGF gene expression during 180 days of follow up.

Table 8. The demographics of patient with low and high level VEGF

Characteristics	Low VEGF	High VEGF
Number	19	13
Gender (F/M)	19/0	13/0
Age (year)	30.0 (34.0-23.0)*	27.0 (34.5-22.5)*
Serum Creatinine (mg/dl)	1.7 (3.9-1.0)*	0.7 (0.9-0.6)*
Protienuria (g/day)	2.3 (4.0-1.1)*	1.5 (3.3-1.1)*
Urinary erythrocyte count (per high power)	15.0 (50.0 -3.0)*	8.5 (36.3-1.0)*
Activity score	8.5 (12.0-4.3)*	1.0 (5.5-0)*
Chronicity score	5 (7.0-1.0)*	2 (2.0-1.0)*

* Inter-quartile range (Q_3-Q_1)

Table 9. The comparisons of renal outcomes (ESRD or doubling serum creatinine) during 6 months of follow up and histological parameters.

Histological parameters	ESRD, n	Doubling serum creatinine, n	ESRD or Doubling serum creatinine, n
Endocapillary proliferation			
- low level	1 (14)	1 (14)	1 (14)
- high level	6 (16)	4 (16)	7 (16)
p value[*]	0.05	0.18	0.02
Crescent formation			
- low level	1 (16)	2 (16)	2 (16)
- high level	6 (14)	3 (14)	6 (14)
p value[*]	0.02	0.50	0.06
Activity index			
- low level	0 (9)	0 (9)	0 (9)
- high level	7 (18)	5 (18)	8 (18)
p value[*]	0.04	0.09	0.02

[*] P value of less than 0.05 was considered significant.