CHAPTER IV

RESULTS

Endothelial cell cytotoxicity induced by Nephrotic Serum

The result of the endothelial cell cytotoxicity test using sera from nephrotic patients revealed (1) that endothelial cell cytotoxicity could be enhanced by sera from patients with both mild nephrosis associated with mesangial proliferation (mean 16.5±5.8% versus control 0.57±1.2%) and severe nephrosis associated with focal segmental glomerulosclerosis (mean 35.3±9.6%); the differences were statistically significant ($p < 0.001$) and (2) that sera from patients with nephrosis associated with focal segmental glomerulosclerosis induced a higher degree of endothelial cell cytotoxicity (Table 1 and Figure 3). Thus, the endothelial cell cytotoxicity test (in vitro) renders a supportive view that endothelial cell injury in vivo is likely to be spontaneously induced in the clinical setting of nephrosis which is supported by the intrarenal hemodynamic study (Table 1.1).

Oxidant and Antioxidant Imbalance

Of the mild form of nephrosis, the initial assessment of oxidant revealed an elevated level of plasma malondialdehyde (MDA) 3.58±0.7 μ M versus 2.48±1 μ M of control. The erythrocyte MDA was also elevated (12.69±2.67 n M versus 7.63±0.93 n M) (Table 2

and Figures 5,6). Of the severe form of nephrosis, the initial value of plasma MDA was 3.71±0.68 μ M. The erythrocyte MDA was 14.18±4.08 n M. In respect to the antioxidant study, there was a significant depletion of antioxidant in both mild and severe forms of nephrosis. The initial values of GSH in mild and severe form of nephrosis were 7.35±1.18 μ mol/g Hb and 6.19±0.8 μ mol/g Hb respectively; versus 9.09±1.66 μ mol/g Hb of normal control. The initial values of vitamin C in mild and severe forms of nephrosis were 1.64±1.43 mg/L and 0.57±0.74 mg/L respectively; versus 4.79±2.5 mg/L of normal control. The initial values of vitamin E in mild and severe forms of nephrosis were 0.11±0.05 m M and 0.10±0.03 n M respectively; versus 0.19±0.05 m M of normal control (Tables 2, 2.1).

Following the therapy with antioxidant and vasodilator, there had been a significant improvement in oxidant and antioxidant status. The plasma and erythrocyte MDA declined to normal level whereas there had been a steady increase in the concentration of GSH, vitamins C and E as depicted in Tables 3, 3.1 and Figure 7-11.

Renal function Study

The initial study of renal function revealed a significant impairment in both mild and severe forms of nephrosis. Of the mild form of nephrosis, the initial creatinine clearance was 88±28

ml/min/1.73m^2 (normal value 120 ml/min/1.73m^2) (Table 4) and the FEMg was 2.9±1% (normal 2.2%). Of the severe form, the initial creatinine clearance was 44.8±24 ml/min/1.73m^2 (Table 4.1) and the FEMg was 5.6±2.6%. The initial glomerular filtration rate in mild and severe forms of nephrosis were 76.5±14.8 ml/min/1.73m^2 and 34±13 ml/min/1.73m^2 respectively. The intrarenal hemodynamic study in mild form of nephrosis revealed a mild impairment. The renal plasma flow (RPF) was 538±146 ml/min/1.73m^2 (normal 600 ml/min/1.73m^2) (Table 5, 5.1, 5.2 and Figure 12), the peritubular capillary flow (PTCF) was 436±142 ml/min/1.73m^2 (normal 480 ml/min/1.73m^2), the efferent arteriolar resistance (RE) was 52±1.9 mm Hg (normal 53 mm Hg). Of the severe form of nephrosis, the RPF was 178±73 ml/min/1.73m^2 (Table 5.3), the PTCF was 144±67 ml/min/1.73m^2, the RE was 15648±8538 dyne.s.cm^{-5} and the PG was 55±2 mm Hg.

Following the therapy with antioxidant and vasodilator, there was a significant improvement in renal function in both forms of nephrosis. Of the mild form, the CCr increased to 128±68 ml/min/1.73m^2 and the FEMg declined to 1.8±0.6%. Of the severe form of nephrosis, the CCr rose to 60±34 ml/min/1.73m^2 and the FEMg declined to 4.1±2.3%. The intrarenal hemodynamics revealed a significant improvement : the RPF rose to 347±75 ml/min/1.73m^2, (Table 5.4, 5.5) the PTCF rose to 284±63 ml/min/1.73m^2, the RE

declined to 5302±161 dyne.s.cm^{-5} and the PG declined to 51±1 mm Hg.

In respect to proteinuria, these combination of vasodilator and antioxidant suppresses the magnitude of proteinuria in nephrosis associated with focal segmental glomerulosclerosis (Table 5.6). This is simply explained by the plausible mechanism that the vasodilator relaxes the efferent arteriolar constriction thereby reduces the large pores (size-selective) of the glomerular dilatation with subsequent reduction in proteinuria, whereas the antioxidant exerts its action to neutralize the oxidative stress that provokes the glomerular endothelial cell cytotoxicity thereby minimizes the glomerular endothelial dysfunction. Therefore, the synergistic action of vasodilator and antioxidant would exert. antiproteinuric as well as vasodilating actions.

Table 1 Endothelial cell cytotoxicity test of 10 patients with mesangial proliferative nephrotic syndrome (MesP-NS), 10 patients with focal segmental glomerulo-sclerosis (FSGS) and 10 controls.

	Control	MesP-NS	FSGS
1	0	8.3	27
2	0	22	55
3	0	17.4	28
4	3	26	32
5	0	21	37
6	0	11.5	32
7	2.7	13.5	31
8	0	10	28
9	0	15	50
10	0	20	33
Mean $\pm$ SD	0.57 $\pm$ 1.20	16.5 $\pm$ 5.8	35.3 $\pm$ 9.6
P value		< 0.001	< 0.001

Table 1.1

illustrated the endothelial cell cytotoxicity and renal plasma flow in idiopathic nephrotic syndrome EC = endothelial cell, MesP-NS = mesangial proliferative nephrosis, NS-FSGS = nephrosis with focal segmental glomerulosclerosis[37]

	EC Cytotoxicity %	Renal Plasma Flow ml/min/1.73m^2
Normal Control	0.6 ± 1.20	595 ± 45
P < .001		
MesP-NS	16.5 ± 5.8	538 ± 146
P < .001		
NS-FSGS	35.3 ± 9.6	178 ± 73

Table 1.2 Illustrated endothelial cell cytotoxicity test during initial and post treatment

Patients	MesP NS		FSGS	
	Initial	Post – Rx	Initial	Post – Rx
1	8.3	0	27	0
2	22	3	55	0
3	17.4	5	28	0
4	26	8	32	7
5	21	3	37	8.5
6	11.5	0	32	0
7	13.5	0	31	0
8	10	0	28	6
9	15	0	50	15
10	20	0	33	5
Mean ± SD	16.5 ± 5.8	1.9 ± 2.8	35.3 ± 9.6	4.1 ± 5.1
P value	< 0.001		< 0.001	

Table 2 Initial value of oxidant and antioxidant studies in mild nephrosis (MesP-NS)

	MesP-NS	Control	P value
Antioxidant GSH (μ mol/g Hb)	7.35 ± 1.18	9.09 ± 1.66	< 0.05
Vitamin C (mg/L)	1.64 ± 1.43	4.79 ± 2.5	< 0.001
Vitamin E (m M)	0.11 ± 0.05	0.19 ± 0.05	< 0.05
Oxidant Plasma MDA (μ M)	3.58 ± 0.70	2.48 ± 1.10	< 0.05
Erythrocyte MDA (n M)	12.69 ± 2.67	7.63 ± 0.93	< 0.05

Table 2.1 Initial value of oxidant and antioxidant in severe nephrosis (FSGS)

	NS-FSGS	Control	P value
Antioxidant GSH (μ mol/g Hb)	6.19 ± 0.18	9.09 ± 1.66	< 0.001
Vitamin C (mg/L)	0.57 ± 0.74	4.79 ± 2.5	< 0.001
Vitamin E (m M)	0.10 ± 0.03	0.19 ± 0.05	< 0.001
Oxidant Plasma MDA (μ M)	3.71 ± 0.68	2.48 ± 1.10	< 0.01
Erythrocyte MDA (n M)	14.18 ± 4.08	7.63 ± 0.93	< 0.001

Table 3 Initial and post-treatment value of oxidant and antioxidant in mild nephrosis (MesP NS)

	MesP-NS		P value
	Initial	Post treatment	
GSH (μ mol/g Hb)	7.35 ± 1.18	8.37 ± 0.77	0.001
Vitamin C (mg/L)	1.64 ± 1.43	4.23 ± 2.31	< 0.001
Vitamin E (m M)	0.11 ± 0.05	0.28 ± 0.08	< 0.001
Plasma MDA (μ M)	3.58 ± 0.70	1.57 ± 0.90	< 0.01
Erythrocyte MDA (n M)	12.69 ± 2.67	7.74 ± 2.13	< 0.001

Table 3.1 Initial and post-treatment value of oxidant and antioxidant in severe nephrosis (FSGS)

	FSGS		
	Initial	Post treatment	P value
GSH (μ mol/g Hb)	6.19 ± 0.18	8.31 ± 1.73	< 0.001
Vitamin C (mg/L)	0.57 ± 0.74	3.64 ± 1.0	< 0.001
Vitamin E (m M)	0.10 ± 0.03	0.29 ± 0.09	< 0.001
Plasma MDA (μ M)	3.71 ± 0.68	1.69 ± 0.86	< 0.001
Erythrocyte MDA (n M)	14.18 ± 4.08	7.54 ± 2.97	< 0.05

Table 4 Initial and post treatment values of renal function in mild nephrosis (MesP-NS)

CCr ml/min/1.73m^2		FE Mg %	
Initial	Post treatment	Initial	Post treatment
Normal	120	2.2	
88 $\pm$ 28	128 $\pm$ 68	2.9 $\pm$ 1	1.8 $\pm$ 0.6
P value	< 0.05	< 0.05	

Table 4.1 Initial and post treatment values of renal function in severe nephrosis (FSGS)

	Initial	Post treatment	P value
CCr ml/min/1.73m^2	44.8 $\pm$ 24	60.6 $\pm$ 34	< .05
FE Mg %	5.6 $\pm$ 2.6	4.1 $\pm$ 2.3	< .05

Table 5 Intrarenal Hemodynamics in Normal Control

Number	GFR ml/min/1.73m^2	RPF ml/min/1.73m^2	PTCF Ml/min/1.73m^2	PG mm Hg	RE Dyne.s.cm^{-5}
1	102	653	551	51	2817
2	102	525	423	51	3250
3	141	600	459	51	2942
4	115	600	485	51	3141
5	120	600	480	51	3162
Mean	116	595	480	51	3062
SD±	±16	±45	±46	±0	±177

Table 5.1 Intrarenal hemodynamics in mild nephrosis (MesP-NS)

Number	GFR ml/min/1.73m^2	RPF ml/min/1.73m^2	PTCF ml/min/1.73m^2	PG mm Hg	RE Dyne.s.cm^{-5}
1	87	523	436	57	3383
2	66	510	444	52	4086
3	114	777	663	51	2142
4	105	506	401	51	3810
5	118	856	738	49	1937
6	97	453	356	52	3810
7	99	439	340	52	4406
8	112	510	398	52	3418
9	90	450	360	51	3841
10	125	369	244	52	6632
11	102	525	423	51	3250
Mean	101	538	436	52	3701
SD±	±16	±146	±142	±1.9	±1233

Table 5.2 Comparison of initial intrarenal hemodynamics between mild nephrosis (MesP-NS) and control

	GFR ml/min/1.73m^2	RPF ml/min/1.73m^2	PTCF ml/min/1.73m^2	PG mm Hg	RE Dyne.s.cm^{-5}
Normal	116 ± 16	595 ± 45	480 ± 46	51	3062 ± 177
MesP-NS	101 ± 16	538 ± 146	436 ± 142	52 ± 1.9	3701 ± 1233
P value	NS	NS	NS	NS	NS

Table 5.3 Initial renal hemodynamics in NS with severe nephrosis (FSGS)

Patients	GFR ml/min/1.73m^2	RPF ml/min/1.73m^2	PTCF ml/min/1.73m^2	PG mm Hg	RE Dyne.s.cm^{-5}
1	44	114	70	57	19367
2	31	100	69	51	20566
3	40	199	159	53	12353
4	58	292	234	55	6036
5	15	86	71	56	33200
6	32	250	218	55	10023
7	28	205	177	58	12073
8	25	181	156	54	11572
Mean	34	178	144	55	15648
SD±	±13	±73	±67	±2	±8538

Table 5.4 Post treatment renal hemodynamics in severe nephrosis (FSGS)

Patients	GFR ml/min/1.73m^2	RPF ml/min/1.73m^2	PTCF ml/min/1.73m^2	PG mm Hg	RE Dyne.s.cm^{-5}
1	51	342	291	50	5373
2	50	235	185	51	8154
3	85	418	333	53	4695
4	57	348	291	51	4842
5	69	391	322	51	4792
6	73	442	369	53	4187
7	72	322	250	53	3324
8	39	278	239	50	6592
Mean	62	347	284	51	5302
SD±	±16	±75	±63	±1	±1611

Table 5.5 The intrarenal hemodynamic study in severe nephrosis (FSGS) during initial and post-treatment.

	RPF ml/min/1.73m^2	PTCF ml/min/1.73m^2	FF	PG mm Hg	RE dyne.s.cm^{-5}	GFR ml/min/1.73m^2
Normal	600	480	0.2	53	3000	120
Initial	178 ± 73	144 ± 67	0.3	55 ± 2	15648 ± 8538	34 ± 13
Post-treatment	347 ± 75	284 ± 63	0.2	51 ± 1	5302 ± 1611	62 ± 16
P value	< .001	< .001	< .01	< .01	< .001	< .01

Table 5.6 Urinary protein in severe nephrosis (FSGS)

Urinary protein (g/day)	
Initial	Post tretmemt
3.45 $\pm$ 1.8	1.26 $\pm$ 1.5
P value < 0.001	

Figure 1 Normal value of intrarenal hemodynamics

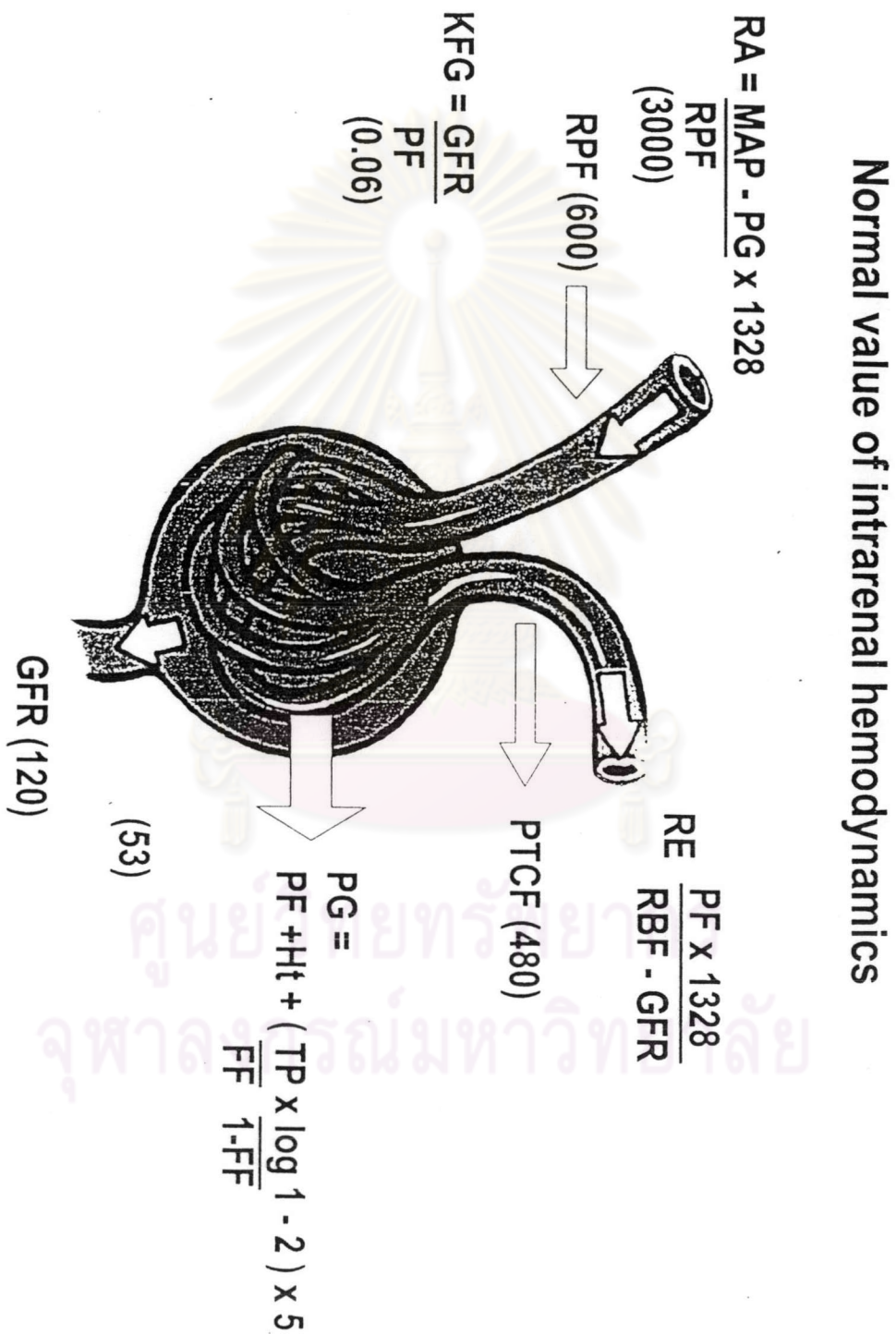

Figure 2 Value of intrarenal hemodynamics in severe nephrosis (FSGS)

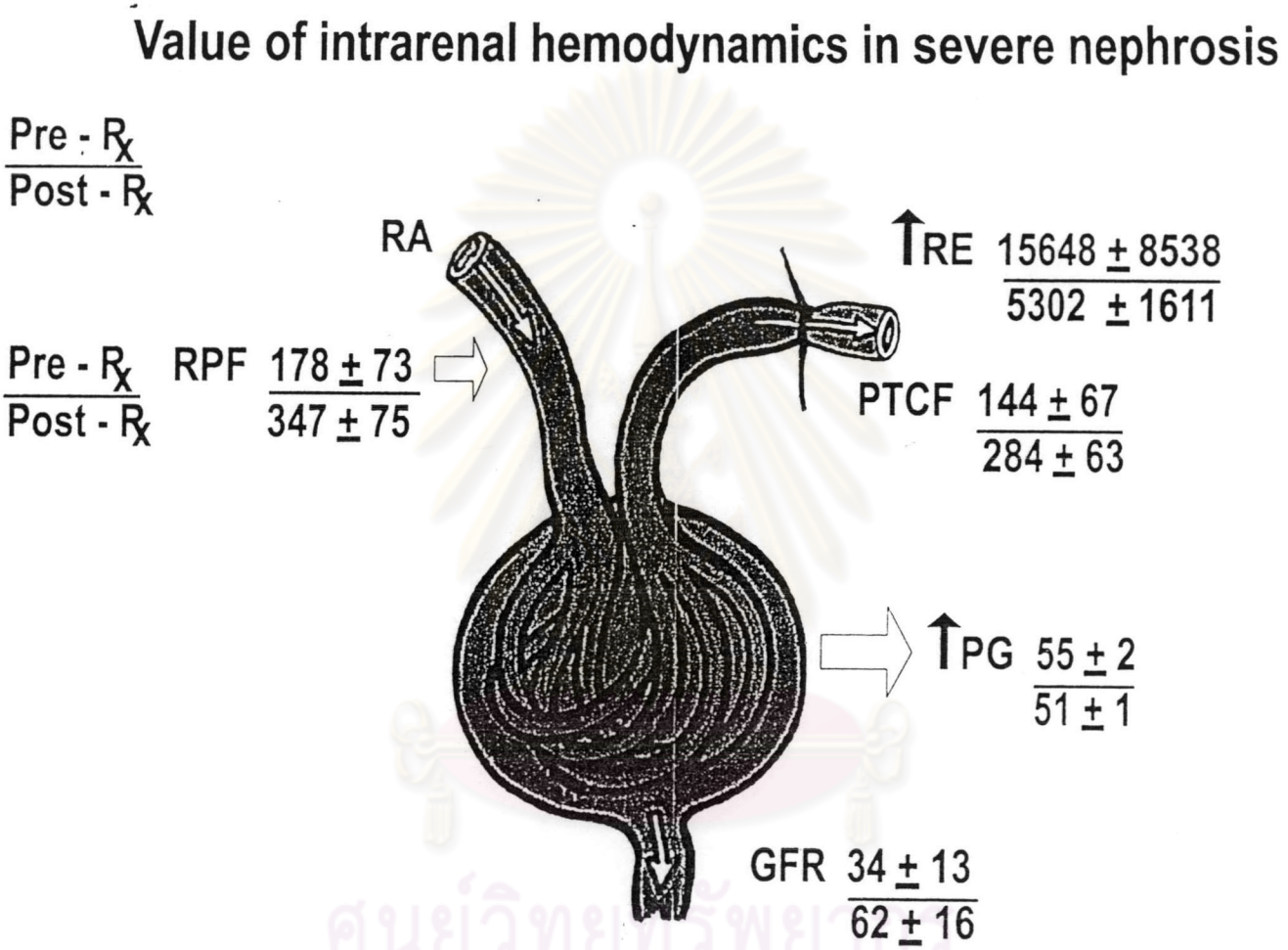

Figure 3 Endothelial cell cytotoxicity in normal compare with severe and mild forms of nephrosis

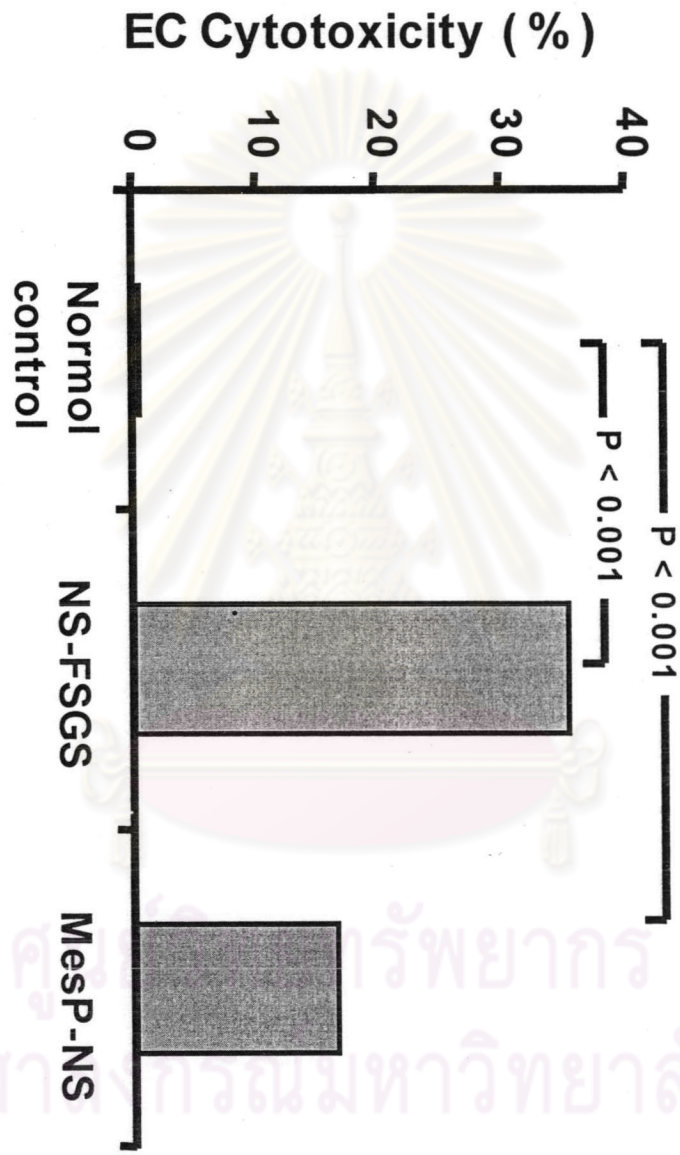

Figure 4 Endothelial cell cytotoxicity compares between initial and post treatment

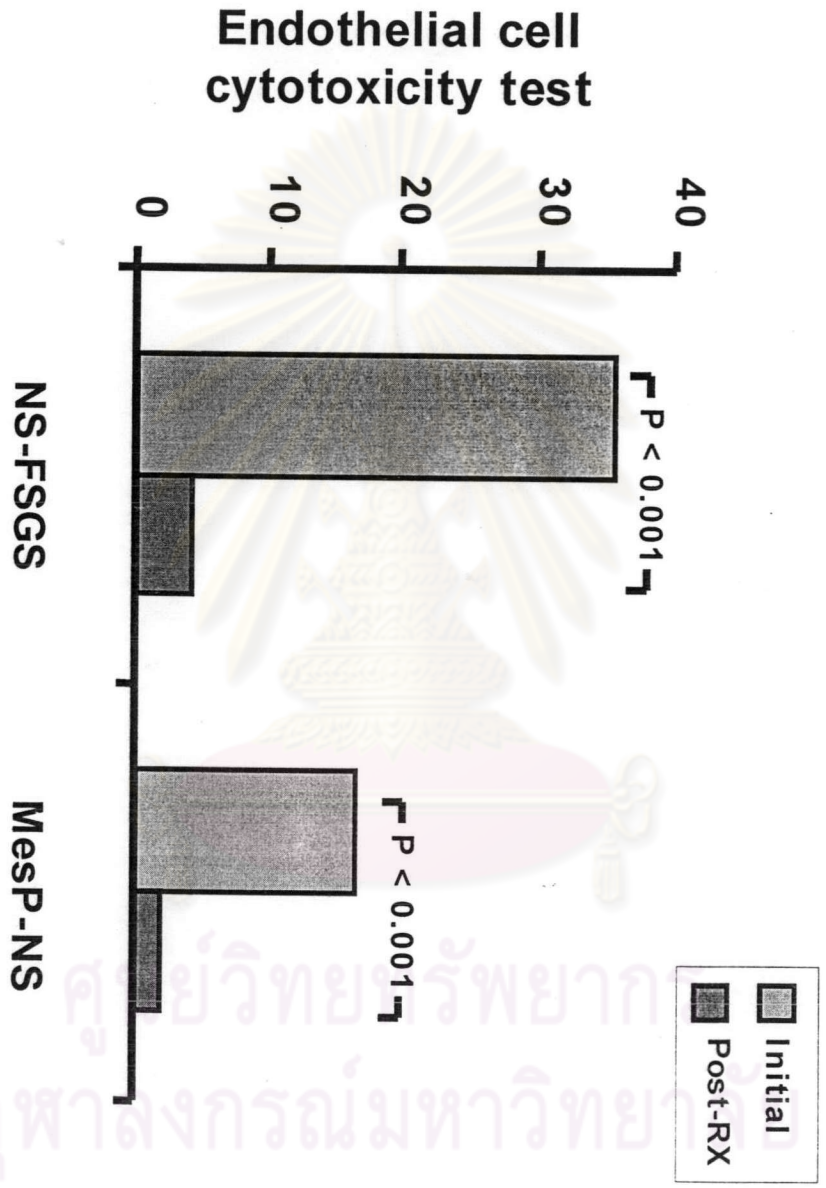

Figure 5 Values of plasma MDA in control compare with severe and mild forms of nephrosis

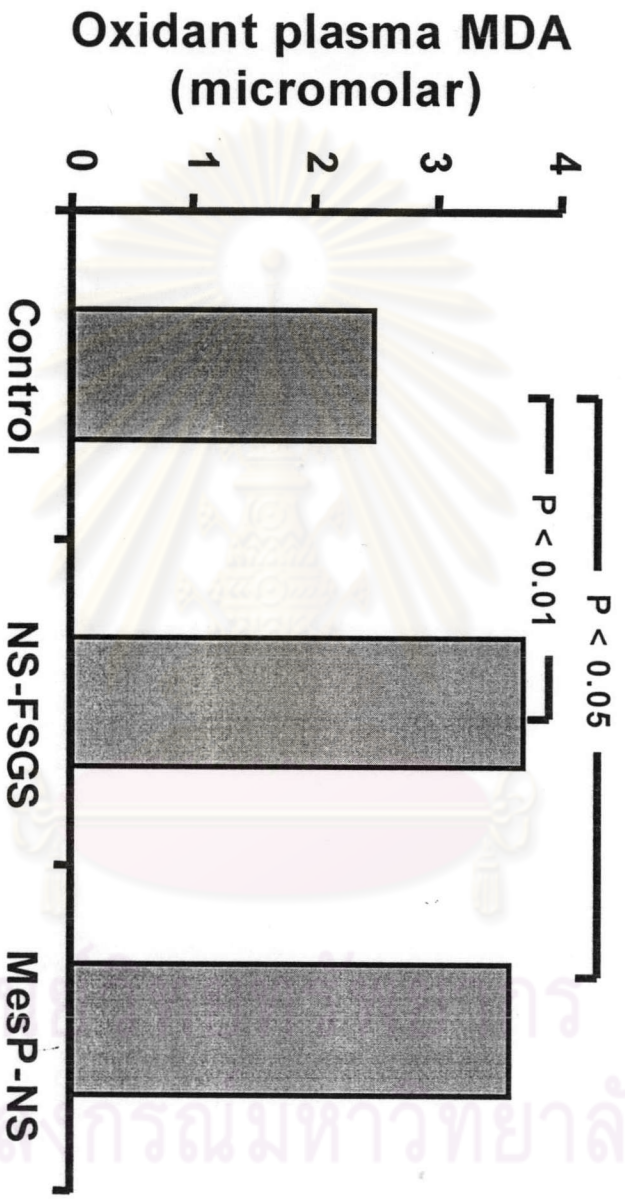

Figure 6 Values of erythrocyte MDA in control compares with severe and mild forms of nephrosis

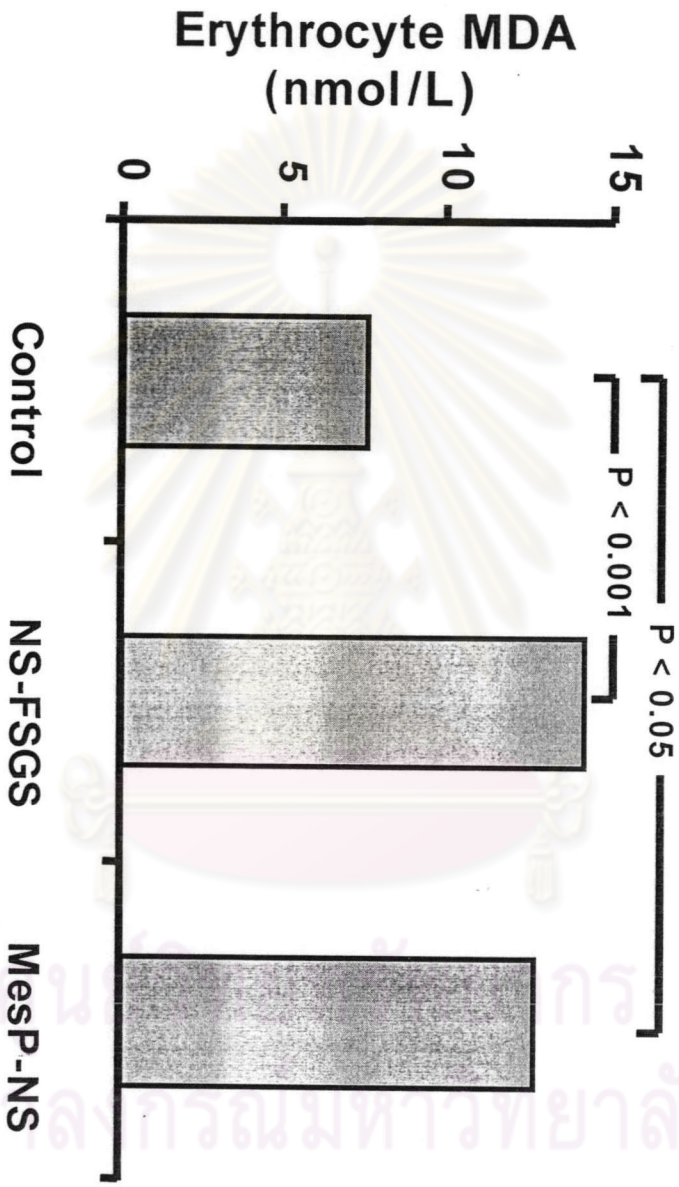

Figure 7 Value of GSH in renal patients compares between initial and post treatment

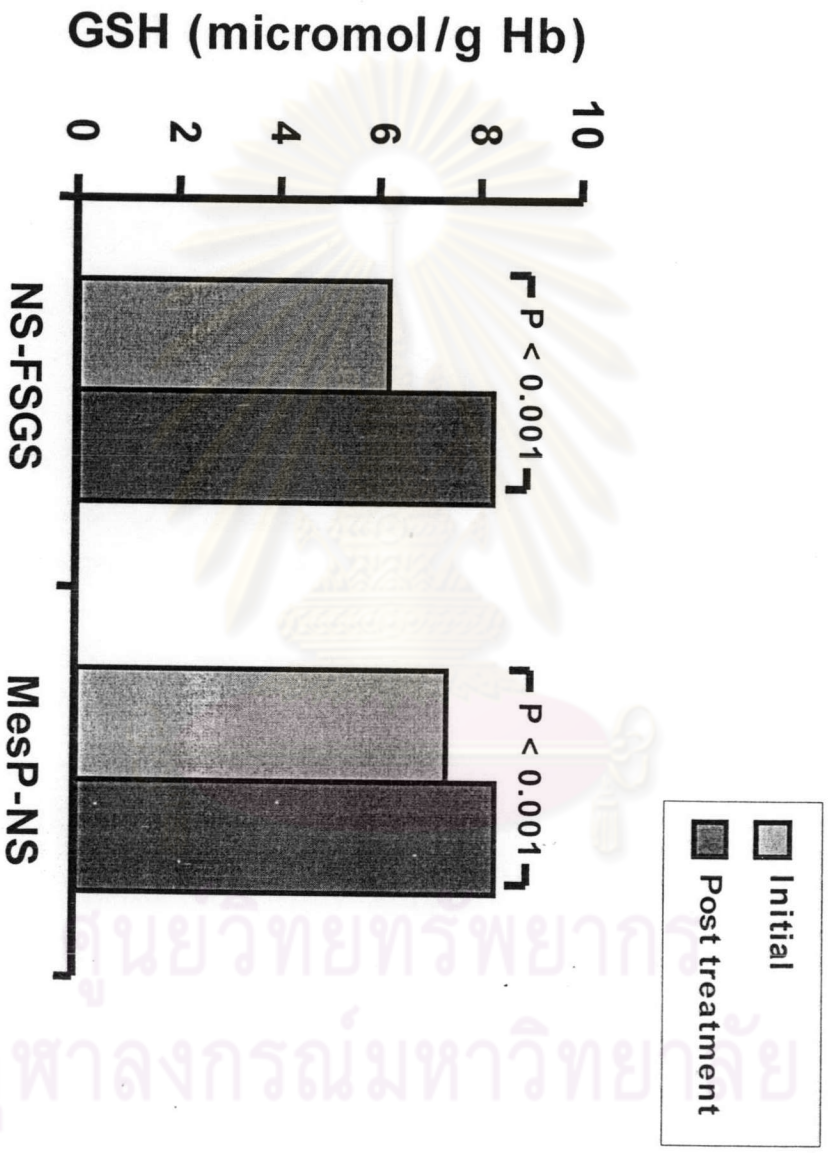

Figure 8 Value of plasma MDA in renal patients compares between initial and post treatment

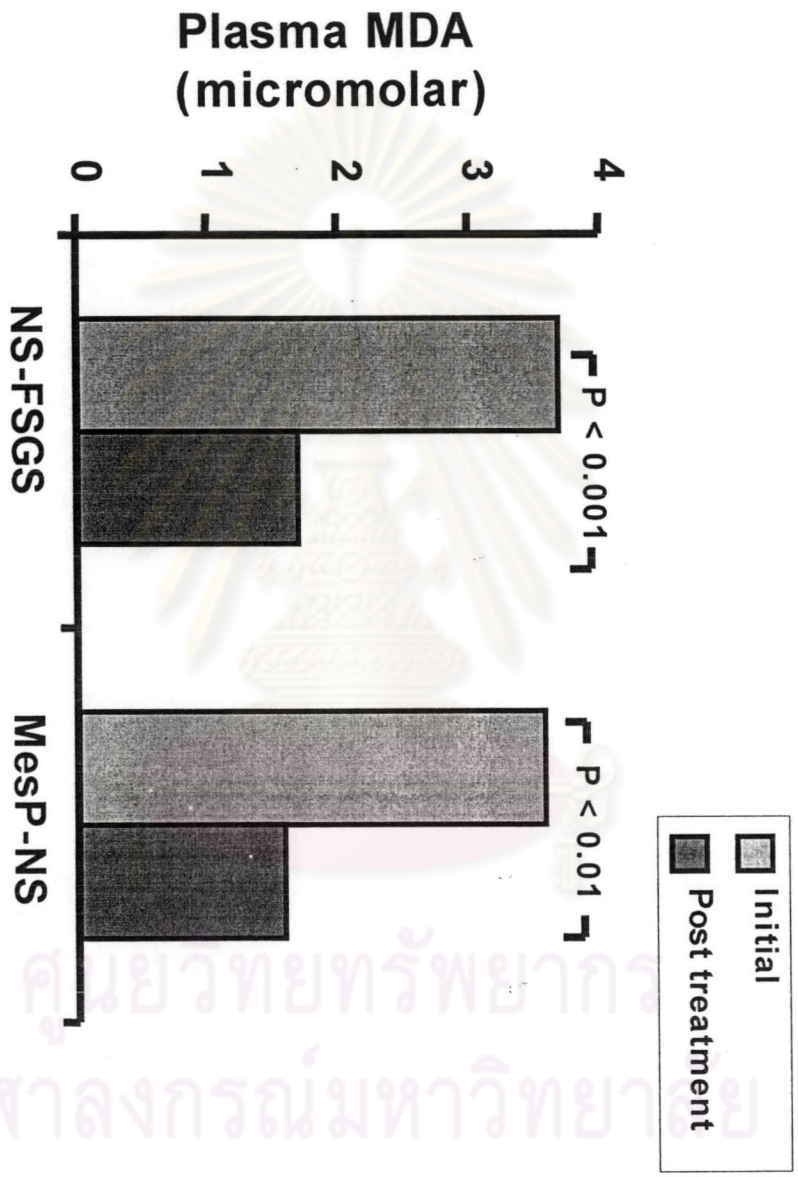

Figure 9 Value of erythrocyte MDA in renal patients compares between initial and post treatment

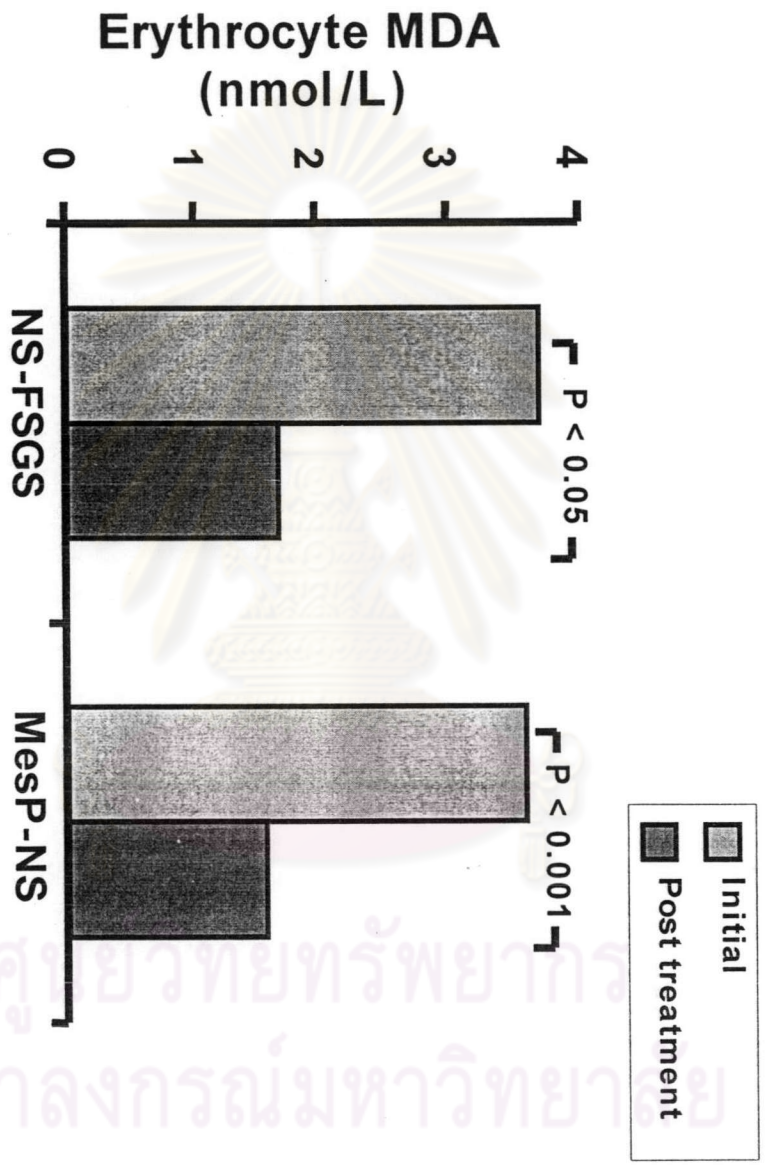

Figure 10 Value of vitamin C in renal patient compares between initial and post treatment

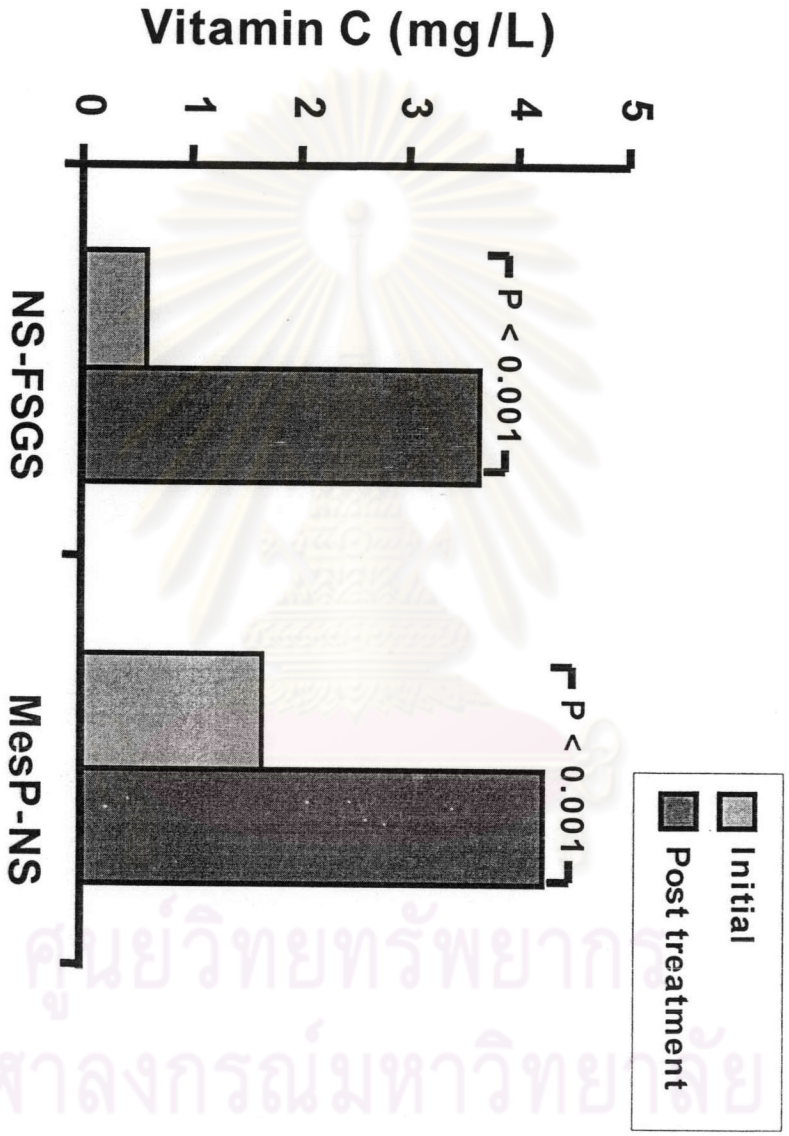

Figure 11 Value of vitamin E in renal patient compares between initial and post treatment

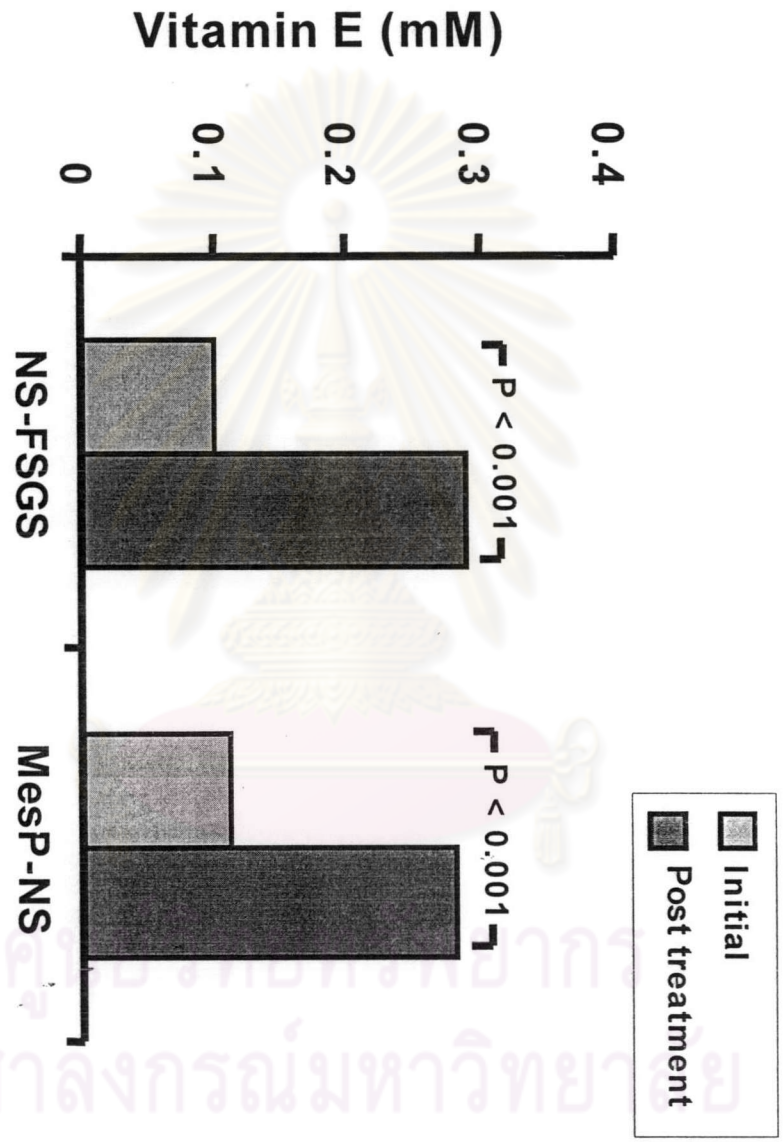

Figure 12 Value of renal plasma flow in renal patients and normal control

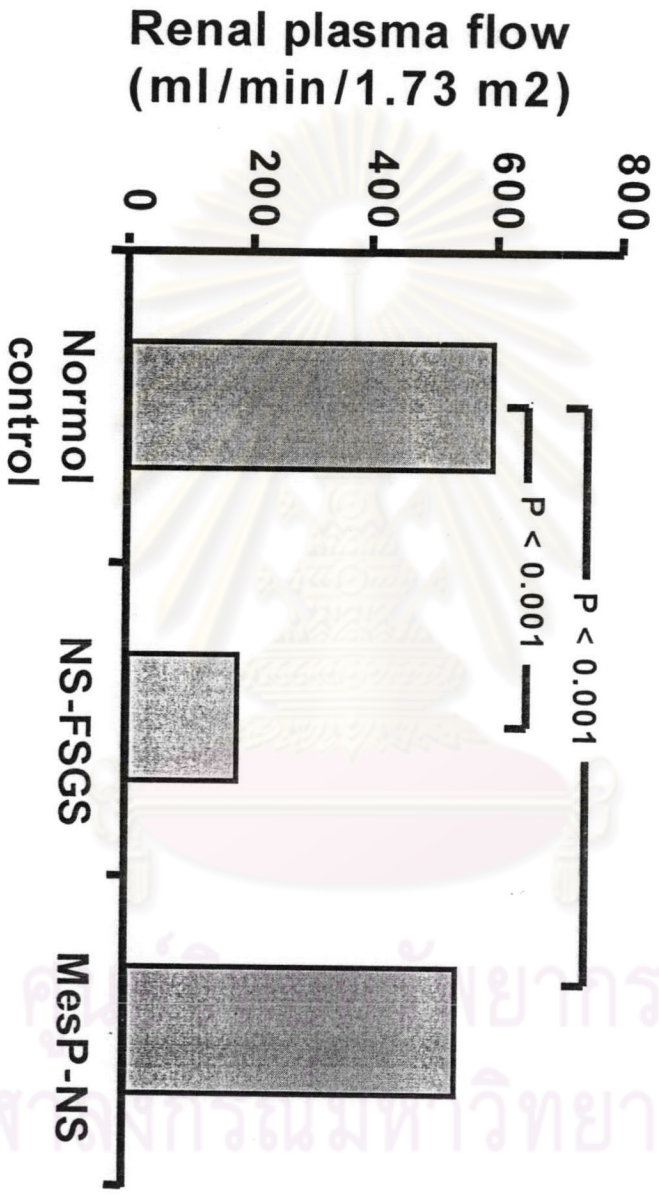

Figure 13 The Spatial Relationship between Renal Perfusion and Nephronal Damage