Geget (2002) 2 (2) : 45 - 60

Original Article

Soluble Thrombomodulin and Serum Plasminogen Activator Inhibitor-1 in Juvenile Lupus Nephritis

Hala Salah, Aysha Badawi*, Sawsan Fadda and Gloria Sidhom*****

Departments of Pediatrics, Internal Medicine Pathology**, Faculty of Medicine, Cairo University; Clinical and Chemical Pathology Basic Science NCR****

ABSTRACT

Background: Systemic lupus erythematosus is a systemic autoimmune disease with multiorgan involvement. It is commonly complicated by autoimmune glomerulonephritis in up to 70% of cases.

Objectives: The objective of this study was to evaluate the clinical significance of soluble thrombomodulin (sTM) and serum plasminogen activator inhibitor–1 (sPAI-1) in juvenile SLE patients and to explore their possible role in lupus nephritis being both related to endothelial cell dysfunction.

Methods: We measured levels of sTM and sPAI-1 in 30 SLE patients and 30 healthy age and sex matched subjects as a control group. Eighteen cases with lupus nephritis, confirmed by renal biopsy were compared with 12 lupus patients without nephritis and control. Among the lupus nephritis cases, six had class I or II nephritis as representative of mild form of lupus nephritis, and twelve with class III, IV or V as representative of severe form of lupus nephritis.

Results: We found that sTM and sPAI-1 were significantly higher in the whole SLE group compared to the control with $p = 0.000$. Cases with lupus nephritis showed significantly higher levels of both sTM and sPAI-1 compared to control $p = 0.000$. Cases without lupus nephritis showed significantly higher levels of sTM and sPAI-1 compared to control with $p < 0.049$ and 0.01 respectively. When we considered the class of lupus nephritis, sTM showed a statistically significant difference between cases with class III, IV & V nephritis versus control and nephritis -ve cases with $p = 0.000$ and < 0.003 respectively. Regarding sPAI-1, statistical analysis revealed significantly higher levels in cases with class III, IV & V compared to control, nephritis -ve, and class I and II patients with $p = 0.000$. Also, sTM was significantly higher in cases with disease activity, proteinuria, endocapillary hypercellularity, interstitial mononuclear cell infiltration, tubular atrophy, and interstitial fibrosis compared to those without, with $p < 0.004$, 0.04, 0.03, 0.04, 0.000 and 0.000 respectively. A statistically significant difference of sPAI-1 was found in lupus patients with and without, proteinuria, urinary casts, endocapillary hypercellularity, interstitial mononuclear cell infiltration, tubular atrophy, glomerulosclerosis, and interstitial fibrosis with $p < 0.027$, 0.048, 0.000, 0.000, 0.001, 0.014, and 0.000 respectively.

Conclusions: In conclusion, our results suggest that dysregulation of the coagulation and fibrinolytic system may be an important mediator of glomerular injury in lupus nephritis and it seems that the more severe and active the kidney lesion, the higher the levels of sTM and sPAI-1, thus, indicating more advanced degree of vasculitis.

INTRODUCTION

Systemic lupus erythematosus is a systemic autoimmune disease with multi-organ involvement. It is commonly complicated by autoimmune glomerulonephritis in up to 70% of cases. Clinical signs of renal disease may vary from mild proteinuria to a rapidly progressive glomerulonephritis with end stage renal failure requiring hemodialysis or kidney transplantation. An interesting feature of lupus glomerulonephritis is the endothelial cell activation and proliferation that accompanies the disease with subsequent intravascular coagulation. This pattern of disease suggests that endothelial cells may

play a central role in the pathophysiology of lupus glomerulonephritis[1].

Thrombomodulin (TM) is a thrombin receptor present on the surface of endothelial cells. It has been reported to be present in blood in a soluble form (sTM) and to be liberated from endothelial cells when these cells are damaged, suggesting that increased blood levels of sTM reflects endothelial cell damage[2].

In this study we aimed to evaluate the clinical significance of sTM as a marker of endothelial cell damage in cases of lupus glomerulonephritis.

Cellular proliferation in the glomeruli with intravascular coagulation usually ends with glomerulosclerosis which is the final pathogenetic pathway leading to progressive loss of renal function in lupus nephritis[3]. Some studies indicate that endothelial cells play an important role in fibrinolysis by secreting tissue plasminogen activator (t-PA) and plasminogen activator inhibitor-1[4]. Dysregulation in glomerular coagulation and fibrinolytic system may play a role in the pathophysiology of lupus nephritis ending with glomerulosclerosis.

Through this study we also tried to explore the possible role of serum plasminogen activator inhibitor-1 (s-PAI-1) in lupus nephritis being also related to endothelial cell dysfunction.

PATIENTS AND METHODS

The study included 30 juvenile SLE patients (24 females and 6 males). The age of the patients ranged from 7 to 20 years; with mean of 13.47 ± 3.36 yr. They fulfilled the American College of Rheumatology (ACR) classification of SLE[5]. The patients were recruited from Collagen Vascular Clinic in Cairo University Pediatric Hospital and outpatient Rheumatology clinic of Internal Medicine Cairo University.

Duration of illness ranged from 1 to 10 years with mean of 4.04 ± 2.5 yr. At the time of blood sampling SLE activity was diagnosed by using SLE disease activity index (SLEDAI)[6].

Thirty healthy controls matched in age and sex with the patients were involved in this work. They had no history of rheumatic disease or chronic illness.

Sampling: Blood was taken from patients and controls after verbal consent by venipuncture, left to clot, centrifuged and sera were stored at -70°C.

Both patients and controls were subjected to the following:

i. Complete history taking.
ii. Thorough clinical and musculoskeletal examinations.
iii. Laboratory investigations included:
- Complete haemogram including platelets count using ABBOT cell counter *(Cell dyn 1600)*.
- ESR was done by Westergren's method.
- Antinuclear antibody (ANA) test was performed with $Heps^2$ cell substrate by kallestod method and developed with fluorescin conjugated antihuman IgG anti-sera.
- Serum creatinine was done by modified Joffe method.
- Quantitative turbidimetric determination of total protein in urine in 24 hours.
- Serum complement c_3 and c_4 was done by radial immunodiffusion.

- Plasma soluble thrombomodulin and serum plasminogen activator inhibitor-1 (PAI-1) were determined by enzyme-linked-immunoassay using IMUBINO thrombomodulin and PAI-1 ELISA kit from American Diagnostica Inc (222 Railroad Ave., P.O. Box 1165, Greenwich, CT 06836-1165).
- Thrombomodulin Kit employs a monoclonal antibody which recognizes the EGF1-EGF2 domains of thrombomodulin with specificity of the capture antibody for native, complexed and truncated thrombomodulin.
- PAI-1 Kit uses a specific mouse monoclonal anti-PAI 1 IgG coated to the microtest wells. The detection level for the kit is 0.5 ng/ml according to assay performance using the standard protocol. The intra- and inter-assay coefficients of variation for precision were 3.6% and 4.2% respectively.

Renal biopsy review:

The biopsies were fixed in 10% formalin, from which paraffin blocks were prepared and multiple serial sections (5 um) were prepared and stained by:

1. Hematoxylin and eosin (HX & E).
2. PAS stain to demonstrate glomerular basement membrane.
3. Masson trichrome stain to detect fibrosis.

Lupus nephritis was classified according to World Health Organization (WHO) classification[7] into class I; normal glomeruli; class II; (lupus mesangiopathy); class III; focal segmental glomerulonephritis (with active necrotizing lesions and/or sclerosis); class IV diffuse proliferative glomerulonephritis (with or without segmental lesions); and class V diffuse membranous glomerulonephritis.

Activity index: The activity index was defined as the sum of individual scores of the following features considered to represent measures of active lupus nephritis: endocapillary hypercellularity (score 0 to 3), cellular crescent (score 0, 2, 4, 6), necrotizing lesion (score 0, 2, 4, 6), hyaline deposit and thrombi (score 0 to 3), interstitial inflammatory cells (score 0 to 3), and glomerular leukocytes (0 to 3). The maximum score was 24 points for the activity index.

Chronicity index: This index consisted of the sum of the following features considered to represent measures of *chronic* irreversible lupus lesions: glomerular sclerosis (score 0 to 3), fibrous crescents (score 0 to 3), tubular atrophy (score 0 to 3), and interstitial fibrosis (score 0 to 3). The maximum score was, 12 points for chronicity index[8].

Statistical Methods

Data were statistically described in terms of range, mean, standard deviation (± SD), median and percentages. Comparison between different groups in the present study was done using Student *t* test for comparing normally distributed parametric data between 2 groups, and analysis of variance (ANOVA) test for more than 2 groups. For comparing non parametric data, Chi square ($\chi 2$) test was performed. Yates correction was used in stead when the frequency is less than 10. Accuracy was represented using the terms sensitivity, specificity, +ve predictive value, -ve predictive value and overall accuracy. Correlation between various

variables were done using Pearson moment correlation coefficient (r) with graphic representation using linear regression graph. A probability value (p value) less than 0.05 was considered significant. All statistical calculations were done using computer programs Microsoft Excel version 7 and SPSS (Statistical Package for the Social Science) statistical program.

RESULTS

Results are displayed in tables (1-8) and figures (1-7).

- Table (1, 2): show the clinical features and the laboratory results of the patients respectively.
- Table (3): shows the details of renal biopsy taken from 18 out of 30 SLE patients with clinical and laboratory features suggesting lupus nephritis.
- Table (4): shows the activity and chronicity indices of the four cases with class IV lupus nephritis.
- Table (5): shows comparison of sTM and sPAI-1 in different study groups. Regarding sTM; in the control group, the mean was 0.74 ± 0.24 ng/ml while in the whole SLE group, it was 1.3 ± 0.50 ng/ml. When we considered cases with nephritis, the mean was 1.47 ± 0.50 ng/ml, while in cases without nephritis it was 1.08 ± 0.42 ng/ml.

Statistical analysis revealed that sTM was significantly higher in the whole SLE group compared to the control with $p = 0.000$. Comparing sTM in control versus those with and without nephritis, there was a highly significant difference between control and nephritis +ve cases with $p = 0.000$ and a significant difference between control and nephritis -ve cases with $p < 0.049$. Also sTM was significantly higher in nephritis +ve compared to nephritis -ve lupus cases with $p < 0.009$.

Regarding sPAI-1, in the control group, the mean was 31.53 ± 4.08 ng/ml while in the whole SLE group, it was 42.9 ± 11.89 ng/ml. When we considered cases with nephritis, the mean was 47.83 ± 12.66 ng/ml, while in cases without nephritis it was 35.5 ± 5.04 ng/ml

Statistical analysis revealed that sPAI-1 was significantly higher in the whole SLE group compared to the control with $p = 0.000$. Comparing sPAI-1 in control versus those with and without nephritis, there was a highly significant difference between control and nephritis +ve cases with $p = 0.000$ and a statistically significant difference between control and nephritis -ve cases with $p < 0.01$. Also sPAI-1 was significantly higher in nephritis +ve compared to nephritis -ve lupus cases with $p < 0.002$.

- Table (6): shows comparison of sTM and sPAI-1 considering the class of nephropathy and other groups. Regarding sTM, the mean was 1.27 ± 0.23 ng/ml in cases with class I & II nephritis, while it was 1.57 ± 0.57 ng/ml in cases with class III, IV & V lupus nephritis.

Statistical analysis revealed no statistically significant difference of sTM between cases with class I & II and nephritis -ve patients or cases with class III IV & V with $p < 0.165$ and 0.144 respectively. However, sTM showed a statistically significant difference between cases with class III, IV & V nephritis versus control and nephritis -ve cases with $p = 0.000$ and

< 0.003 respectively. Comparing sTM in control versus cases of class I & II nephritis revealed a significant difference with p < 0.002.

Regarding sPAI -1, the mean was 32.8 ± 9.3 ng/ml in cases with class I & II nephritis, while it was 55.33 ± 4.91 ng/ml in cases with class III, IV & V lupus nephritis. Statistical analysis revealed no statistically significant difference of sPAI-1 between cases with class I & II and control or nephritis -ve cases with p < 0.310 and 0.218 respectively. However, sPAI-1 showed a highly significant difference between cases with class I & II nephritis and those with class III, IV and V nephritis, and cases with class III, IV & V and nephritis -ve cases with p = 0.000. A highly significant difference was found comparing the control group with cases with class III, IV & V nephritis with p = 0.000.

- Table (7): shows comparison of sTM levels in SLE cases with different parameters.

There was no statistically significant difference of sTM in lupus patients with or without hypertension, urinary casts, hematuria, pyuria, hypocomplementemia or glomerulosclerosis with p < 0.213, 0.127, 0.335, 0.216, 0.061 and 0.358 respectively. Also, soluble thrombomodulin did not show any significant difference between cases kept on HQ ± low dose steroids and those who needed more aggressive regimen including immunosuppressive drugs with p < 0.4. A statistically significant difference of sTM was found in lupus patients with and without disease activity, proteinuria, endocapillary hypercellularity, interstitial mononuclear cell infiltration, tubular atrophy, and interstitial fibrosis with p < 0.004, 0.04, 0.03, 0.04, 0.000 and 0.000 respectively.

- Table (8): shows comparison of sPAI-1 levels in SLE cases with different parameters. There was no statistically significant difference of sPAI-1 in lupus patients with or without hypertension, disease activity, hematuria, pyuria, or hypocomplementemia with p < 0.499, 0.103, 0.194, 0.116 and 0.153 respectively. Also, there was not any significant difference in the levels of sPAI-1 between cases treated with low dose steroids versus those who were treated with more aggressive immuno-suppression with p < 0.1. A statistically significant difference of sPAI-1 was found in lupus patients with and without, proteinuria, urinary casts, endocapillary hypercellularity, interstitial mononuclear cell infiltration, tubular atrophy, glomeru-losclerosis, and interstitial fibrosis with p < 0.027, 0.048, 0.000, 0.000, 0.001, 0.014 and 0.000 respectively.

Fig. (1-4) show different histological features of lupus nephritis.

Fig. (5-7) show sensitivity, specificity and accuracy of both thrombomodulin and PAI-1 with histological features of lupus nephritis.

Table 1: Clinical features of the patients

Parameter	SLE patients n = 30 Mean ± SD or n (%)
Age (yr.)	13.47 ± 3.36
M : F	1:4
Duration (yr.)	4.05 ± 2.5
Hypertension	8 (26.67)
Malar rash	26 (86.67)
Alopecia	26 (86.67)
Arthritis and/or arthralgia	23 (76.67)
Serositis	5 (16.67)
Carditis	11 (36.67)
Vasculitis	14 (46.67)
Raynaud's phenomenon	2 (6.67)
Pulmonary	3 (10 .00)
CNS	9 (30 .00)
Active disease	18 (60.00)
Treatment: • HQ ± low dose ST • HQ + high dose ST ± IS	 5 (16.67) 25 (83.33)

HQ: Hydroxychloroquin. ST: Steroids. IS: Immunosuppressives

Table 2: Laboratory findings of the patients

Parameter	SLE patients n = 30 Mean ± SD or n (%)
ESR mm/h	48.03 ±38.16
No. (%) of anemia (HB < 12)	26 (86.66)
No. (%) of thrombocytopenia (< 150.000/ml³)	2 (6.66)
No. (%) of leucopenia (< 4000/ml³)	5 (16.66)
No. (%) of lymphopenia (< 1500/ml³)	10 (33.33)
Serum creatinine mg/dl	0.77 ± 0.86
No. (%) of proteinuria (> 0.5 gm /24 h)	15 (50)
No. (%) of granular casts	12 (40)
No. (%) of pyuria	11 (36.67)
No. (%) of hematuria	10 (33.33)
No. (%) of +ve ANA	24 (80)
No. (%) of +ve anti ds-DNA	17 (56.67)
No. (%) of +ve hypocomplementemia C3 < 90 IU/ml	10 (33.33)

Table 3: Histological features of lupus nephritis

Parameter	Number (%) 18 (100%)
Endocapillary hypercellularity	10 (55.56)
Hyaline thrombi	2 (11.11)
Mononuclear cell interstitial infilteration	11 (61.11)
Leukocyte infiltration of the glomerulus	2 (11.11)
Cellular crescents	0 (0)
Glomerular sclerosis	5 (27.78)
Fibrous crescents	2 (11.11)
Tubular atrophy	6 (33.33)
Interstitial fibrosis	8 (44.44)

Table 4: Activity and chronicity indices of class IV lupus nephritis biopsies

Patient No.	Activity index	Chronicity Index
1	8/24	4/12
2	7/24	2/12
3	9/24	6/12
4	8/24	4/12

Table 5: Comparison of soluble thrombomodulin and serum plasminogen activator inhibitor-1 in different study groups

Parameter	Control (N=20)	Overall Patients (N=30)	Nephritis +ve (N=18)	Nephritis -ve (N=12)	P1=	P2=	P3<	P4<
sTM (ng/ml)	0.74±0.24	1.30±0.50	1.47±0.50	1.08±0.42	0.000	0.000	0.049	0.009
sPAI-1 (ng/ml)	31.53±4.08	42.9±11.89	47.83±12.66	35.5±5.04	0.000	0.000	0.01	0.002

P1: control vs overall cases.
P2: control vs nephritis +ve cases.
P3: control vs nephritis –ve cases.
P4: nephritis +ve vs nephritis –ve cases.

Table 6: Comparison of soluble thrombomodulin and plasminogen activator inhibitor-1 considering the class of nephropathy and other groups

Parameter	Control (N=20)	Nephritis -ve (N=12)	I & II (N=6)	III & IV & V (N=12)	P1<	P2=	P3<	P4<	P5<
sTM (ng/ml)	0.74±0.24	1.08±0.42	1.27±0.23	1.57±0.57	0.002	0.000	0.165	0.003	0.144
sPAI-1 (ng/ml)	31.53±4.08	35.5±5.04	32.8±9.3	55.33±4.91	0.310	0.000	0.218	0.000	0.000

P1: control vs class I & II lupus nephritis.
P2: control vs class III & IV & V lupus nephritis.
P3: nephritis –ve vs class I & II lupus nephritis.
P4: nephritis –ve vs class III & IV & V lupus nephritis.
P5: class I & II vs class III & IV & V lupus nephritis.

Table 7: Comparison of soluble thrombomodulin levels in SLE patients with different parameters

Parameter	No. of cases	Mean ± SD (ng/ml)	p <
• Hypertension	8	1.44 ± 0.57	
• No hypertension	22	1.25 ± 0.48	0.213
• Disease activity	18	1.5 ± 0.52	
• No disease activity	12	1.01 ± 0.32	0.004
• Hypocomplementemia	10	1.51 ± 0.61	
• No hypocomplementemia	20	1.20 ± 0.42	0.061
• Proteinuria	15	1.46 ± 0.48	
• No proteinuria	15	1.15 ± 0.49	0.04
• Urinary casts	12	1.43 ± 0.56	
• No urinary casts	18	1.22 ± 0.46	0.127
• Hematuria	10	1.36 ± 0.59	
• No hematuria	20	1.28 ± 0.47	0.335
• Pyuria	11	1.40 ± 0.57	
• No pyuria	19	1.25 ± 0.46	0.216
• Endocapillary hypercellularity	10	1.54 ± 0.58	
• No endocapillary hypercellularity	20	1.19 ± 0.43	0.03
• Interstitial infiltration	11	1.51 ± 0.56	
• No interstitial infiltration	19	1.18 ± 0.44	0.04
• Glomerulosclerosis	5	1.38 ± 0.56	
• No glomerulosclerosis	25	1.29 ± 0.50	0.358
• Tubular atrophy	6	2.03 ± 0.26	
• No tubular atrophy	24	1.12 ± 0.37	0.000
• Interstitial fibrosis	8	1.82 ± 0.48	
• No interstitial fibrosis	22	1.12 ± 0.37	0.000
• HQ ± low dose ST	5	1.25 ± 0.59	
• HQ + high dose ST ± IS	25	1.31 ± 0.50	0.4

HQ: Hydroxychloroquin . ST: Steroids . IS : Immunosuppressives

Table 8: Comparison of serum plasminogen activator inhibitor-1 levels in SLE patients with different parameters

Parameter	No. of cases	Mean ± SD (ng/ml)	p <
• Hypertension	8	43.25 ± 14.58	
• No hypertension	22	42.8 ± 11.1	0.499
• Disease activity	18	45.2 ± 13.1	
• No disease activity	12	39.5 ± 9.4	0.103
• Hypocomplementemia	10	46.1 ± 14.2	
• No hypocomplementemia	20	41.3 ± 10.6	0.153
• Proteinuria	15	47.1 ± 12.6	
• No proteinuria	15	38.7 ± 9.9	0.027
• Urinary casts	12	47.3 ± 12.7	
• No urinary casts	18	39.9 ± 10.7	0.048
• Hematuria	10	45.6 ± 13.2	
• No hematuria	20	41.6 ± 11.3	0.194
• Pyuria	11	46.4 ± 12.8	
• No pyuria	19	40.9 ± 11.2	0.116
• Endocapillary hypercellularity	10	52.6 ± 11.9	
• No endocapillary hypercellularity	20	38.1 ± 8.6	0.000
• Interstitial infiltration	11	55.3 ± 5.1	
• No interstitial infiltration	19	35.7 ± 8.1	0.000
• Glomerulosclerosis	5	53.4 ± 6.5	
• No glomerulosclerosis	25	40.8 ± 11.7	0.014
• Tubular atrophy	6	55.7 ± 5.4	
• No tubular atrophy	24	39.7 ± 10.9	0.001
• Interstitial fibrosis	8	55.75 ± 3.6	
• No interstitial fibrosis	22	38.2 ± 10.3	0.000
• HQ ± low dose ST	5	36.6 ± 8.9	
• HQ + high dose ST ± IS	25	44.2 ± 12.2	0.10

HQ: Hydroxychloroquin. ST: Steroids. IS: Immunosuppressives

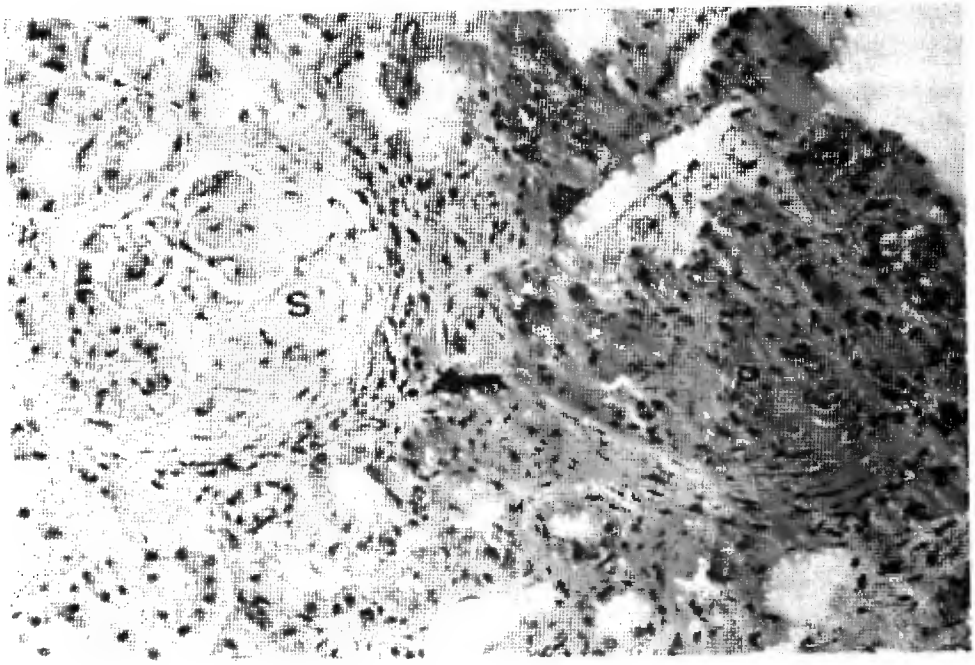

Fig. (1): A case of lupus nephritis class III showing segmental proliferation (P) in one glomerulus and sclerosis (S) in another one. There are interstitial fibrosis and inflammatory infiltrates (arrow) [Masson trichrome x 200].

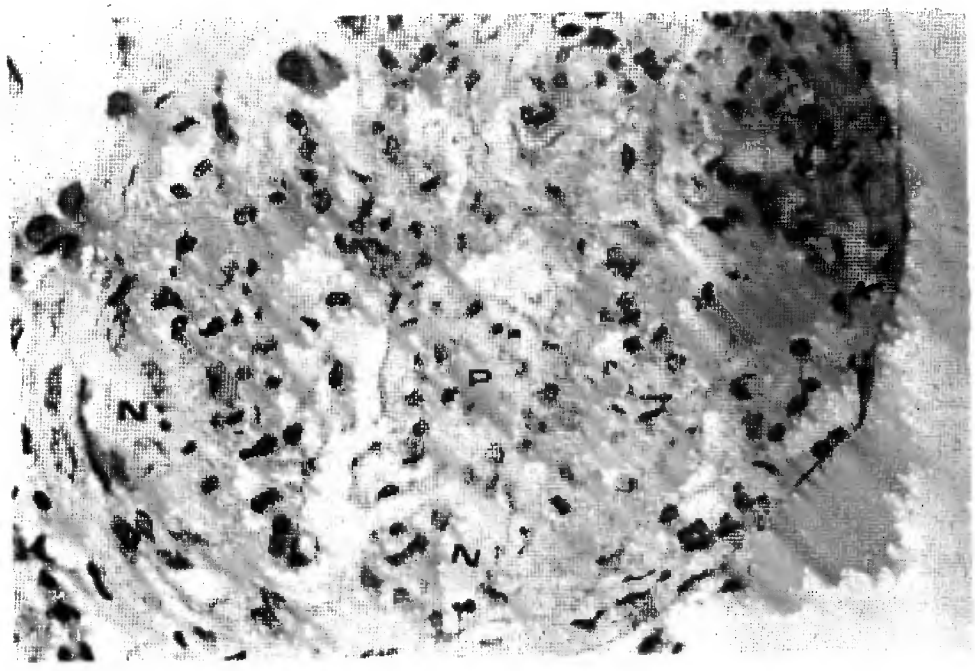

Fig. (2): A case of lupus nephritis class IV showing endocapillary cellular proliferation (P), fibrinoid necrosis (N) and thick glomerular basement membrane (arrow) [H &E x 400].

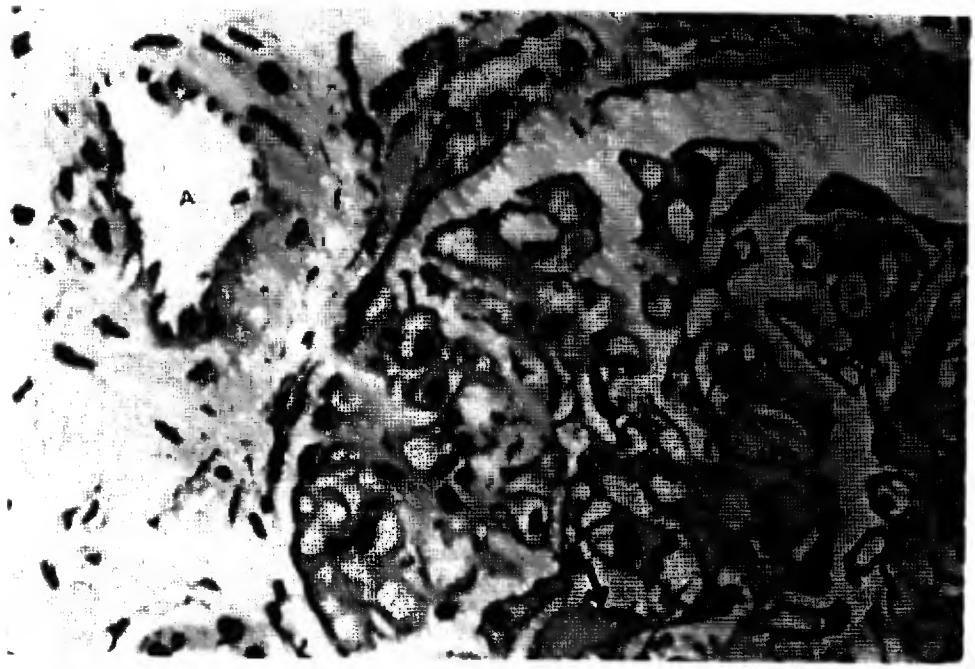

Fig. (3): A case of lupus nephritis class V showing wire loop formation (arrow) and thick adjacent artery (A) [H &E x 400].

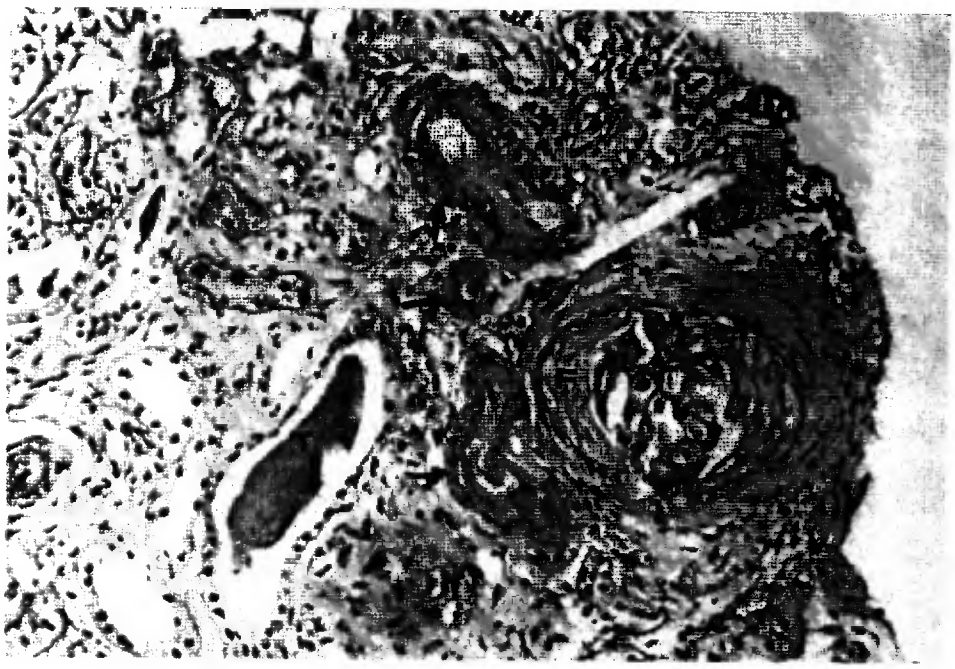

Fig. (4): A case of lupus nephritis with fibrous crescent (F) compressing the tuft, thick walled arterioles (arrows) and tubular atrophy with cast [PAS x 200].

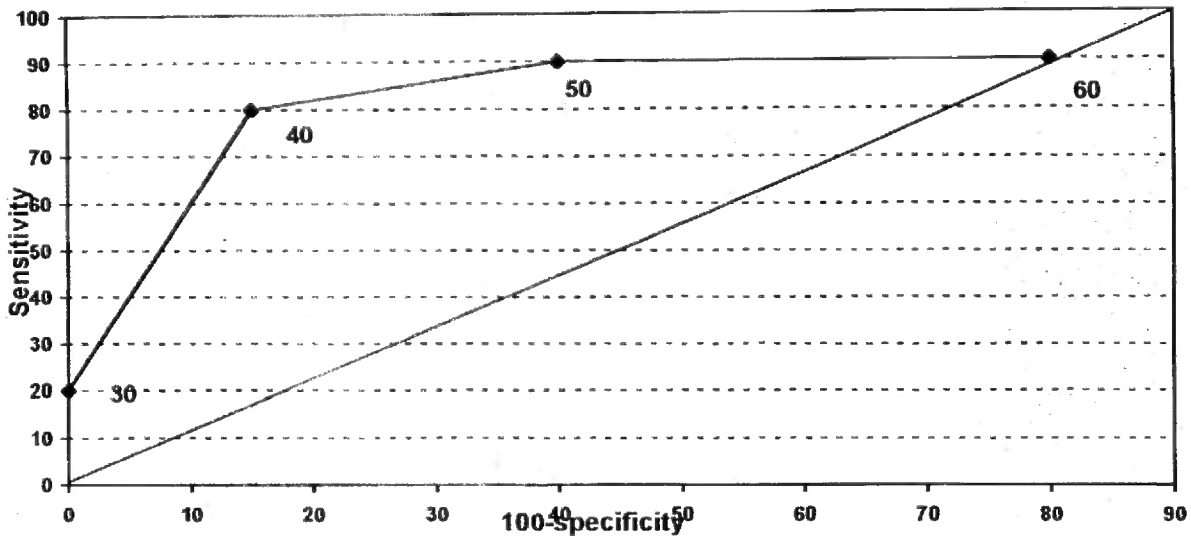

Fig. (5): ROC-curve shows sensitivity, specificity and accuracy of PAI-1 and endocapillary hypercellularity of lupus nephritis at different cut off levels. When the cut off level of PAI-1 is 50 ng/ml the sensitivity is 80%; the specificity is 85% and accuracy is 83.3%.

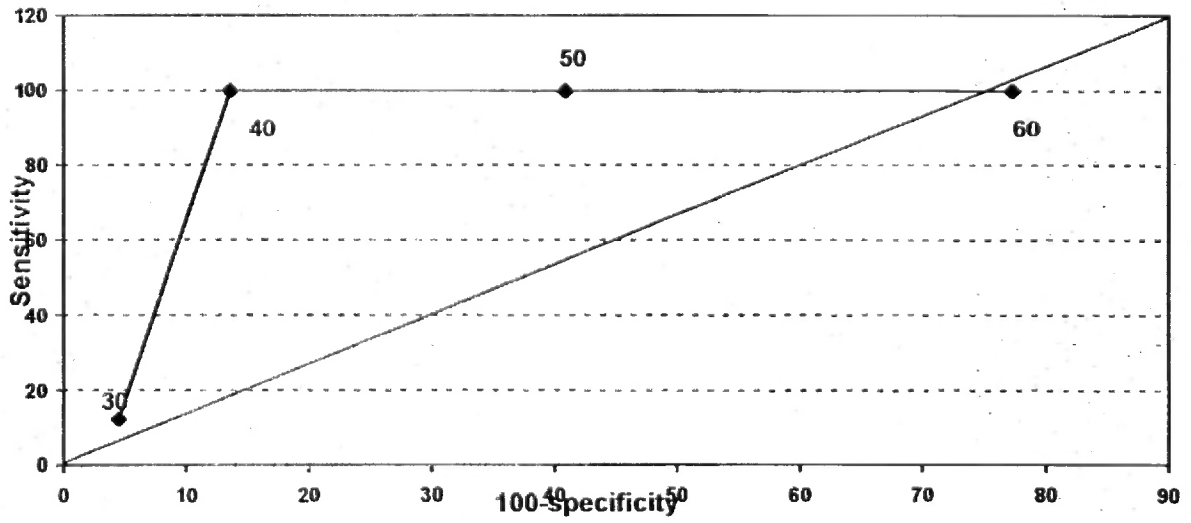

Fig. (6): ROC-curve shows sensitivity, specificity and accuracy of PAI-1 and interstitial fibrosis of lupus nephritis at different cut off levels. At cut off level of PAI-1 of 50 ng/ml the sensitivity is 100%, the specificity is 86.4% and accuracy is 90%.

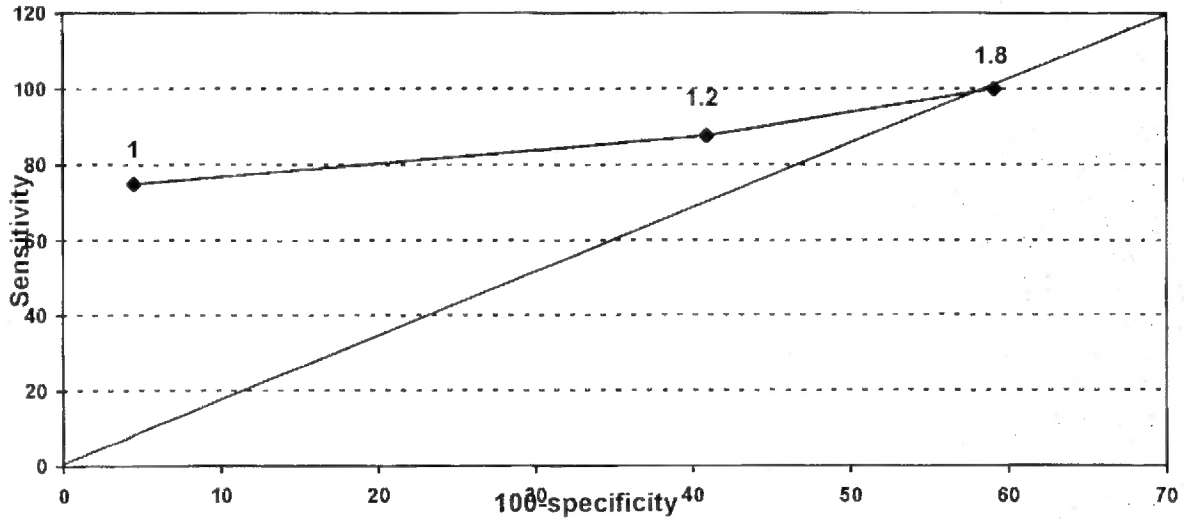

Fig. (7): ROC-curve shows sensitivity, specificity and accuracy of sTM and interstitial fibrosis at different cut off levels. As the cut off level of sTM is 1.8 ng/ml, the sensitivity is 75% & the specificity is 95.5% and the accuracy is 90%.

DISCUSSION

Pathophysiologically SLE is characterized by an immune complex vasculitis and increased cytotoxicity of polymorphonuclear neutrophils (PMN) to endothelial cells[9]. Soluble thrombomodulin (sTM) was established as a marker of endothelial cell damage in vasculitides[10]. Different studies reported increased sTM in conditions where there is wide spread damage to the vascular endothelium[11]. In the present study there was a statistically significant rise of sTM levels in the entire group of SLE patients, lupus patients without nephritis and lupus cases with nephritis compared to control $p = 0.000$, < 0.049 and $= 0.000$ respectively. Also, cases with lupus nephritis showed significantly higher levels of sTM compared to cases without nephritis $p < 0.009$. These results conform with those reported by ***Frijns et al. 2001***[12] who found a remarkable difference in sTM concentration when lupus nephritis cases were compared with lupus patients without nephritis $p < 0.001$. ***Tomura et al 1994***[13] demonstrated in an immuno-fluorescence study an increased expression of thrombomodulin along the capillary wall in the glomeruli of patients with lupus nephritis, suggesting enhanced production of thrombomodulin in glomerular endothelial cells in those patients. Based on these findings, enhanced expression of thrombomodulin by glomerular endothelial cells and vasculitis that leads to vascular endothelial cell injury are important determinants of increased sTM levels that are found in the current study.

Statistical analysis of sTM levels in relation to the class of nephritis showed a highly significant elevation of sTM in cases with class III, IV & V compared to control and cases of SLE without nephritis with $p = 0.000$ and < 0.003 respectively. Also, sTM levels were significantly higher in cases with disease activity, proteinuria, endocapillary hypercellularity, interstitial

mononuclear cell infiltration, tubular atrophy and interstitial fibrosis than in cases without $p < 0.004$, 0.04, 0.03, 0.04, = 0.000, and 0.000 respectively. So, it seems that the more severe and active the kidney lesion, the higher the levels of sTM thus, indicating more advanced degree of vasculitis.

Glomerulosclerosis and interstitial fibrosis are the final common pathways that lead to progressive renal failure in cases of SLE[3]. Current evidence supports the view that fibrosis of the kidney is a consequence of peturbation of the normal balance between extracellular matrix synthesis and its degradation[4]. Although the molecular basis for this process remains unclear, it has been proposed that a dysfunction in glomerular coagulation and proteolysis may cause persistent fibrin deposition in the intraglomerular capillaries and may promote further glomerular injury by causing capillary thrombosis and subsequent inflammatory cell infiltration[15]. Glomerular fibrin deposits also, have a direct cytotoxic effect on mesangial cells[16]. Recent studies indicated that endothelial cells play an important role in fibrinolysis by secreting tissue plasminogen activator (tPA) which converts plasminogen into plasmin[17]. Plasmin is a serine proteinase that activates a latent matrix metalloproteinase and itself has a limited matrix degrading activity[18]. Endothelial cells secrete large amounts of plasminogen activator inhibitor-1 (PAI-1) in response to IL1, TNF, and lipopolysaccharides stimulation[17]. Increased PAI-1 expression in the glomerulus inhibits fibrin clearance at sites of coagulation and causes disease progression.

To explore the possible role of dysregulation of the plasmin protease system in the development and progression of lupus nephritis, we measured serum sPAI-1 levels in SLE patients. Comparison of sPAI-1 in the control versus the entire group of SLE patients, cases with and cases without lupus nephritis revealed statistically significant differences with $p = 0.000$, 0.000 and < 0.01 respectively. Comparison of serum sPAI-1 levels in lupus patients with and without nephritis showed significantly higher values in cases with nephritis with $p < 0.002$. These results conform with those of ***Moll et al., 1995***[19], who reported a marked induction of PAI-1 gene expression in three different strains of lupus prone mice (NZB X NZW) F1, BXSB and MRL lpr/lpr. The glomerular localization of PAI-1 mRNA as indicated by in situ hybridization experiments suggests that PAI-1 may be synthesized by inflammatory cells infiltrating glomeruli, glomerular mesangial cells and/or endothelial cells as demonstrated by ***Keeton et al. 1995***[4] in MRL lpr/lpr mice. Cytokines locally released by infiltrating inflammatory cells may play a role in the induction of PAI-1. In this regard, ***Newman et al., 1990***[20] demonstrated concomitant increase in glomerular expression of TGF-β, a cytokine known to be a potent inducer of PAI-1 biosynthesis in vitro and in vivo. ***Moll et al., 1995***[19] demonstrated that the increases in PAI-1 and TGF-β mRNA correlated very well with the severity of glomerular lesions in lupus prone mice.

The current study showed that sPAI-1 levels were significantly higher in lupus cases with proteinuria compared to those

without ($p < 0.04$). Also, there was significantly higher serum levels of PAI-1 in lupus nephritis patients with class III, IV & V compared to lupus cases with class I & II ($p < 0.007$). However, there was no statistically significant difference in serum levels of PAI-1 in class I & II lupus nephritis cases versus nephritis negative lupus cases or control with ($p < 0.388$ and 0.357) respectively. This means that in milder forms of lupus nephritis there was no significant rise of sPAI-1 compared to control and nephritis negative cases thus, suggesting that this important inhibitor of fibrinolysis may indeed contribute to the pathogenesis and progression of lupus nephritis.

Statistical study of serum sPAI-1 levels in relation to individual histologic features in renal biopsies taken from lupus nephritis cases showed a statistically significant higher levels in cases with endocapillary hypercellularity, mononuclear cell interstitial infiltration, glomerulosclerosis, tubular atrophy, and interstitial fibrosis compared to those without ($p < 0.018$, 0.007, 0.02, 0.01, and 0.004) respectively. The results of this study agree with the report published by ***Wang et al.; 2001***[17] who studied the effect of a single base pair insertion/ deletion 4G/5G polymorphism of the PAI-1 gene on the development and severity of lupus nephritis. They found that patients with the 4G/4G genotype had the highest levels of PAI-1 activity and this was strongly associated with a greater nephritis activity than those of the 4G/5G or 5G/5G genotypes.

It is postulated that overexpression of PAI-1 results in an imbalance between plasminogen/plasmin system. This in turn, causes persistent fibrin deposition in the intraglomerular capillaries and promotes further glomerular injury by causing capillary thrombosis with subsequent inflammatory cell infiltration[15]. Another obvious consideration is the possibility that PAI-1 itself might function as a chemo-attractant, even though, a cellular receptor for PAI-1 has not yet been described[14].

In conclusion, our results suggest that dysregulation of the coagulation and fibrinolytic system may be an important mediator of glomerular injury in lupus nephritis. Further studies are recommended to search for the exact role of PAI-1 in the cascade of events that transforms the interstitial cellularity to a fibrosis-promoting phenotype and to address the different cytokines in this process which may lead to a novel therapeutic approach for retarding the progression of lupus nephritis.

REFERENCES

1. **Kant, K.S.; Pllak, V.E. and Dosekun, A. (1985):** Lupus nephritis with thrombosis and abnormal fibrinolysis: effect of ancord. J. Lab. Clin. Med. 105 : 77-88.
2. **Ishii, H.; Uchiyama, H. and Kazama, M. (1991):** Soluble thrombomodulin antigen in conditioned medium is increased by damage of endothelial cells. Thromb. Haemost; 65: 618-623.
3. **Tomooka, S.; Border, W.A.; Marshall, B.C. and Noble, N.A. (1992):** Glomerular matrix accumulation is linked to inhibition of the plasmin protease system. Kidney Int. 42: 1462-1469.
4. **Keeton, M.; Ahn, C. and Eguchi, Y. (1995):** Expression of type 1 plasminogen activator inhibitor in renal tissue in murine lupus nephritis. Kidney Int. 47: 148-157.

5. **Tan, E.N.; Cohen, A.S.; Fries, J.F.; Masi, A. T. and Winchester, R.J. (1982):** The revised criteria for the classification of systemic lupus crythematosus. Arthritis Rheum. 25: 1271-1277.
6. **Bombardier, C.; Gladmann, D.P.; Urowity, M.B. and Caron, P. (1992):** The committee on prognosis studies in SLE: Derivation of SLEDAI: a disease activity index for lupus patients .Arthritis Rheum 35:630-640.
7. **Churg, J.; Bernstein, J. and Glassock, R.J. (1995):** Renal disease: Classification and Atlas of Glomerular Diseases (2nd ed). New York, Tokyo, Igaku–Shoin Medical Publisher, 1995, pp 151-179.
8. **Austin, H,A.; Mvenz, L.R.; Joyce, K.M.; Automon Ych, T.A. and Balou, J.E. (1984):** Diffuse proliferative lupus nephritis, identification of specific pathological features affecting renal outcome Kidney Int. 25: 689-695.
9. **Hashimoto, Y.; Nakano, K. and Yoshinoya, S. (1992):** Endothelial cell destruction by polymorphonuclear leukocytes incubated with sera from patients with SLE. Scand. J. Rheumatol. 21: 209 – 214 .
10. **Sawada, K.; Yamamoto, H. and Yago, H. (1992):** A simple assay to detect endothelial cell injury; measurement of released thrombomodulin from cells. Exp. Mol. Pathol.; 57: 116-123.
11. **Mercie, P.; Seigneur, M.; Constans, J. and Conri, C. (1997):** Assay of thrombomodulin in systemic diseases. Rev. Med. Interne. 18: 126 - 31.
12. **Frijns, R.; Fijnheer, R. and Schiel, A. (2001):** Persistent increase in plasma thrombomodulin in patients with a history of lupus nephritis: Endothelial cell activation markers. J. Rheumatol. 28: 514-519.
13. **Tomura, S.; Deguchi, F.; Marumo, F. and Aoki, N. (1994):** Enhanced presence of thrombomodulin in the glomeruli of lupus glomerulonephritis. Clin Nephrol. 41: 205- 10 .
14. **Oda, T.; Jung, Y.O.; Kim, H.S. and Cai, X. (2001):** PAI-1 deficiency attenuates the fibrogenic response to ureteral obstruction. Kid. Internat. 30: 587-596.
15. **Rondeau, E.; Mougenot, B. and Lacave, R. (1990):** Plasminogen activator inhibitor in renal fibrin deposits of human nephropathies. Clin. Nephrol. 33: 55-60 .
16. **Tsumagari, T. and Tanaka, A. (1984):** Effects of fibrinogen degradation products on glomerular mesangial cells in culture. Kidney Int. 26: 712-718.
17. **Wang, A.Y.; Poon, P.; Lai, F.M. and Yu, L. (2001):** Plasminogen activator inhibitor-1 gene polymorphism 4G/4G genotype and lupus nephritis in Chinese patients. Kidney Int. 59: 1520-1528.
18. **Vassalli, J.D.; Sappino, A.P. and Belin, D. (1991):** The plasminogen activator/plasmin system. J. Clin. Invest. 88: 1067- 1072.
19. **Moll, S.; Menoud, P.A.; Fulpius, T. and Pastore, Y. (1995):** Induction of plasminogen activator inhibitor type 1 in murine lupus-like glomerulonephritis. Kidney Int. 48: 1459-1468.
20. **Newman, M.J.; Lane, E.A.; Iannotti, A.M.; Nugent, M.A. and Pepinsky, R.B. (1990):** Characterization and purification of a secreted plasminogen activator inhibitor (PAI-1) induced by transforming growth factor–β1 in normal rat kidney (NRK) cells: Decreased PAI-1 expression in transformed NRK cells. Endocrinology. 126: 2936-2946.