Original article

Assessment of plasma and urinary transforming growth factor beta 1 (TGF-β1) in children with lupus nephritis

Background: *Kidney disease is one of the most serious manifestations of systemic lupus erythematosus (SLE). Despite the improvement in the medical care of SLE in the past two decades, the prognosis of lupus nephritis remains unsatisfactory. Transforming growth factor- β1 (TGF-β1) is an immuno-suppressive cytokine, as it inhibits T and B cell proliferation and NK cell cytotoxic activity .*

Objective: *The aim of this study was to assess serum and urinary TGF- β1 levels in children with SLE and their possible role in the renal involvement and activity of the disease.*

Study design: *This cross sectional study was conducted in Nephrology Unit of Pediatric Department, plus Outpatient Clinic of Rheumatology Department , Zagazig University Hospital during the year of 2010.*

Methods: *Twenty-five pediatric patients with SLE were randomly selected and classified according to into 2 groups: Group (I): included 13 patients presented with urinary abnormalities and/or disturbed renal function(active nephritis): 5 males, 8 females. Their mean age was 9.7±2.53 years and the mean disease duration was 2.46±1.4 years. Group (II): included 12 patients presented by lupus without nephritis : 5 males,7 females. Their mean age was 9.9±2.1 years and the mean disease duration was 2.41±0.9 years. Control group(group III): Twenty healthy children of matched age and sex served as a control group included 8 males ,12 females. Their mean age was 10.0±2.3 years.*

Results: *There was no significant difference among studied patients groups regarding age, sex , disease duration and lupus therapy (p>0.05). There was a significant difference between both groups regarding urinary albumin and serum creatinine (2.76±0.97 and 1.96±0.84 mg/dl respectively) ,while there was a high significant difference between them regarding C3 (47.3±12.5 and 76.6±6.6 mg/ml respectively) and anti double stranded DNA (anti-dsDNA) (80.7±32.8 and 26.8±4.5 IU/ml respectively). Plasma TGF- β1 showed significantly lower levels in patients with active nephritis relative to other groups, while urinary TGF- β1 levels were significantly high in SLE patients either with active or silent nephritis when compared with the control group. Plasma TGF- β1 showed a highly significant positive correlation with C3 and a highly significant negative correlation with serum creatinine, urinary albumin, anti dsDNA and SLE disease activity index (SLEDAI) score. While, urinary TGF- β1 had a significant negative correlation with C3 and a high significant positive correlation with anti-dsDNA and SLEDAI score.*

Conclusion: *Low plasma TGF β1 level and increased urinary TGF β1 excretion denotes active renal affection in children with SLE .*

Keywords: SLE , nephritis , TGF- β1.

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INTRODUCTION

Systemic lupus erythematosus (SLE) is a generalized autoimmune disorder characterized by T and B cell hyperactivity with autoantibodies against numerous cell components and deposition of immune complexes. The disease has a multifactorial pathogenesis with genetic, hormonal and environmental components[1,2].

In autoimmune diseases, infiltration with T cells or deposition of autoantibody-containing immune complexes in target organs, such as kidneys, causes early inflammatory lesions. The early immune mediated injury is believed to trigger a series of events, including complement activation, chemokine production, further inflammatory cell infiltration, and inflammatory cytokine release, eventually resulting in deposition of extracellular matrix[3].

Detection of antinuclear antibodies in serum is one of the most frequent laboratory tests for diagnosis of SLE. Anti dsDNA antibodies are considered a hallmark of SLE and anti-Sm antibodies have a significant association with lupus nephritis. However, not all anti-dsDNA antibodies can deposit in the kidney, nor has it been possible to replicate the disease of lupus nephritis with passive transfer of anti-dsDNA antibodies[4].

Transforming growth factor- β1(TGFβ1) affects differentiation, inhibits proliferation and induces apoptosis of B and T cells, and it is an important costimulator generating regulatory CD4 +CD25+ and CD8+ T cells, that help to maintain tolerance against self and non-self antigens. It was originally isolated from platelets but has also been found in cell cultures of monocytes/macrophages and renal mesangial cells[5,6].

Normally, the release of TGF- β1 is controlled by a feedback mechanism when the healing process has been completed. However, if its release is not switched off, extracellular matrix components are accumulated and tissue fibrosis occurs. Renal TGF-β1 is a key mediator of glomerular and tubulointerstitial pathology in renal diseases[7].

The aim of this study was to assess plasma and urinary TGF- β1 levels in children with SLE and their possible role in the renal involvement and activity of the disease.

METHODS

This study was a cross-sectional one conducted in Pediatric department, Nephrology Unit plus Outpatient Clinic of Rheumatology Department, Zagazig University Hospital during the year of 2010.

Patient groups

Included 25 pediatric patients fulfilling the 1997 revised classification criteria of American Rheumatism Association for SLE[8]. The clinical disease activity for each patient with SLE was scored using the SLE disease activity index (SLEDAI)[9]. Patients were designated to be disease-active when their major clinical manifestations were present or SLEDAI was>3[19]. Patients presented with renal disorders were subjected to renal biopsy for histopathologic classification using WHO classification system[10]. The criterion for the diagnosis of renal disorder included the presence of: a)persistent proteinuria 0.5 gm/24h (or more than +3 dipstick reaction for albumin), or b) cellular casts including: red blood cell, granular, renal tubular or mixed[11].

According to presence of clinical manifestations of renal affection, abnormal urinary findings and renal biopsy staging, patients were classified into two groups:

Group (I): included 13 patients with active nephritis: 5 males, 8 females. Their mean age was 9.7±2.53 years and the mean disease duration was 2.46±1.4 years. Renal biopsy results denoted that: 2 (15.4%) patients were in class I, 4 (30.8%) in class II, while class III and IV were present in 3 and 4 patients respectively.

Group (II): included 12 patients without clinical or laboratory renal affection (lupus without nephritis): 5 males, 7 females. Their mean age was 9.9±2.1 years and the mean disease duration was 2.41±0.9 years.

Control group (III): Twenty healthy children of matched age and sex served as a control group. It included: 8 males,12 females. Their mean age was 10.0±2.3 years).

Ethical approval was obtained from the local research ethics committee and parents of all subjects gave an informed written consent prior to the study.

All patients were subjected to:

- Thorough clinical examination
- Laboratory investigations including:

1. Complete blood count (SYSMEX K1000 Automated hematology analyzer).
2. Liver and Kidney function (ADVIA 1650 Autoanalyzer Siemens medical solution diagnostic).
3. Complete urine analysis.
4. Detection of serum anti-ds DNA by ELISA technique (Diagnostic Automation Inc).
5. Detection of serum C3 by Radial immuno diffusion (Binding Site LtD KIT , UK).
6. TGF-β1 levels (in plasma and urine) were measured by ELISA .

Sample collection

Blood samples were obtained by sterile venipuncture , two mls of blood were delivered into an EDTA tube and centrifuged to obtain plasma for detection of TGF-β1.

Serum samples were separated from three ml of blood and kept at -70°C used for assay of anti-ds DNA and C3.

Aseptically collected random urine sample was taken and centrifuged to remove particulates, was used for assay of TGF-β1.

TGF β1 assay used monoclonal antibody as capture antibody and biotinylated polyclonal antibody as detection antibody (BD Biosciences Pharmingen, San Diego, US). The assay specifically measures active TGF-β1 forms. To measure total TGF-β1, biologically latent TGF was activated by acid-treatment. For this purpose, samples were diluted

with four volumes of Phosphate-buffered saline. Samples were then incubated in the presence of 1N HCl , then neutralized with equal volume of 1N Na OH .ELISA analysis was performed in 96-well plates following the instructions of the manufacturer . Recombinant human TGF-β1 (BD Biosciences Pharmingen, San Diego, US) was used as a standard .

Statistical methods

The data of the study was statistically analysed by SPSS (Statistical Package for Social Science) (Version 15). Difference in the level of TGF-β1 between different groups was assessed by the use of ANOVA test. Spearman correlation coefficient was used for correlation of the quantitative variables. Chi squared test, student t-test and Mann Whitney (MW) test were used for comparison between the studied groups. P-value<0.05 was considered significant.

RESULTS

Table (1) shows demographic data of studied groups. There was no significant difference among studied groups regarding age , sex, disease duration and therapy (p>0.05).

Table (2) shows clinical characteristics of 25 patients with SLE. Most of the patients (72%) presented with cutaneous manifestations, 60% presented with arthralgia or arthritis, 52% had renal manifestations (hematuria, persistent proteinuria, disturbed renal functions). Nervous system, gastrointestinal, cardiovascular and pulmonary involvements were present in 24, 20, 16 and 8 % of patients respectively. Five patients (20%) were presented with malaise and fever, while 3 children (12%) had splenomegaly, hepatomegaly or lymphadenopathy.

Table (3) shows laboratory markers and SLEDAI score in lupus patients with active and without nephritis. There was a significant difference between both groups regarding urinary albumin and serum creatinine (2.76±0.97 vs 1.96±0.84 mg/dl). While there was a high significant difference between them regarding C3 (47.3±12.5 vs 76.6±6.6 mg/ml) and anti dsDNA (80.7±32.8 vs 26.8±4.5 IU/ml). Group (I) showed a significantly higher SLEDAI score than group II (38.5±11.4 vs 28.6±9.4).

Table (4) shows plasma and urinary TGF-β1 in studied subjects. There was a highly significant difference between them regarding both plasma and urinary TGF-β1. Plasma TGF-β1 level showed significantly lower levels in patients with active nephritis relative to other groups. While, urinary TGF-β1 levels were significantly high in SLE patients either with active or silent nephritis when compared with the control group.

Table (5) shows correlation between plasma TGF-β1 and other laboratory parameters. There was a highly significant positive correlation with C3 and a high significant negative correlation with serum creatinine, urinary albumin, anti ds-DNA and SLEDAI score.

Table (6) shows correlation between urinary TGF-β1 and other laboratory parameters. There was a highly significant negative correlation with C3 and a highly significant positive correlation with anti-dsDNA and SLEDAI score. While, there was no significant correlation with serum creatinine, urinary albumin.

Table 1. Demographic data of studied groups.

	Group (I) Lupus with active nephritis (n =13)	**Group (II) Lupus without nephritis (n =12)**	**Group (III) Control (n = 20)**	**Statistical test**	**P**
Sex n (%)					
Male	5(38.5)	5(41.7)	8(40)	χ^2=0.03	0.9
Female	8(61.5)	7(58.3)	12(60)		
Age (years)					
Mean±SD	9.7±2.53	9.9±2.1	10.0±2.3	F=0.06	0.9
Range	7-15	7-14	7-15		
Disease duration (years)					
Mean±SD	2.46±1.4	2.41±0.9		t=0.09	0.9
Range	1-5	1-4	-		
Lupus Therapy n (%)					
S	4(30.8)	4(33.3)			
S+I	5(38.5)	3(25)	-	χ^2=0.66	0.8
C+I	2(15.4)	2(16.7)			
S+C+I	2(15.4)	3(25)			

p>0.05 non significant, * p<0.05 significant, ** p≤0.001 highly significant

S: steroid (Prednisolone, Medrol), I: immunosuppressive drugs (Imuran, Sandimmune), C: cytotoxic drugs (Methotrexate)

Table 2. Clinical characteristics of 25 patients with SLE.

Symptom	Number of patients	%
Cutaneous and mucous membrane lesions	18	72
Arthralgia and/or arthritis	15	60
Renal manifestations (hematuria, persistent proteinuria, disturbed renal functions)	13	52
Central and/or peripheral nervous system involvement	6	24
Gastrointestinal disorders	5	20
Malaise, fever	5	20
Cardiovascular disorders and Raynaud's phenomenon	4	16
Lymphadenopathy, splenomegaly, Hepatomegaly	3	12
Pneumonitis and/or pleurisy	2	8

Table 3. Laboratory criteria and SLEDAI score in patients with active and silent lupus nephritis.

	Group (I) Lupus with active nephritis (n =13)	Group (II) Lupus without nephritis (n =12)	Statistical test	p
S. creatinine (mg/dl) Mean±SD Range	 2.76±0.97 1.3-4.7	 1.96±0.84 0.7-3.5	t=2.21	0.03*
Anti-dsDNA (IU/ml) Median Mean±SD Range	 81.5 80.7±32.8 37-127	 27.5 26.8±4.5 18-33	MW=17.28	0.001**
C3 (mg/ml) Mean±SD Range	 47.3±12.5 25-66	 76.6±6.6 65-88	t=7.26	0.001**
Urinary albumin 0 +1 +2 +3 +4	 0…..0 2….15.4 4….30.8 4….30.8 3….23.1	 4….33.3 4….33.3 4….33.3 0….0 0….0	χ^2=11.65	0.02*
SLEDAI score Mean ± SD Range	 38.5±11.4 21-53	 28.6±9.4 20-46	t=2.36	0.02*

* p<0.05 significant,** p≤0.001 highly significant

Table 4. Plasma and urinary TGF- β1 in the studied subjects:

	Group (I) Lupus with active nephritis (n =13)	Group (II) Lupus without nephritis (n =12)	Group (III) Control (n = 20)	F	p
P. TGF-β1 (pg/ml) Mean ± SD Range	 625.8±114.7[b] 448.7-778	 815±126 645.9-1012.3	 896.7±114.8 567.3-1155	16.7	<0.001**
U. TGF-β1 (ng/ml) Mean ± SD Range	 120.3±34 [a,b] 70.8-168.8	 102.9±26.7 [c] 62-144.6	 81.6±10.2 62.8-98.5	10.7	<0.001**

* p<0.05 significant,** p≤0.001 highly significant
[a]Significant difference between active and silent nephritis.
[b]Significant difference active nephritis and the control.
[c] Significant difference silent nephritis and the control.

Table 5. Correlation between plasma TGF-β1 and other laboratory parameters.

P. TGF-β1 (pg/ml)	**r**	**p**	**Sig.**
Anti-dsDNA (IU/ml)	-0.85	<0.001**	HS
C3 (mg/ml)	0.88	<0.001**	HS
S. creatinine (mg/dl)	-0.44	<0.05	Sig.
Urinary albumin	-0.55	<0.001**	HS
SLEAI score	-0.83	<0.001**	HS

Table 6. Correlation between urinary TGF-β1 and other laboratory parameters.

U. TGF-β1 (ng/ml)	r	p	Sig.
Anti-dsDNA (IU/ml)	0.71	<0.001**	HS
C3(mg/ml)	-0.64	<0.001**	HS
S. creatinine (mg/dl)	0.15	>0.05	N.sig.
Urinary albumin	0.26	>0.05	N.sig.
SLEAI score	0.84	<0.001**	HS

*p>0.05 non significant, p<0.05 significant,** p≤0.001 highly significant

DISCUSSION

Nephritis in SLE is one of the most important causes of morbidity and mortality. One-third of patients with systemic lupus erythematosus (SLE) nephritis have flares despite the best treatment and still progress to end-stage renal disease[12,13].

TGF-β1 plays a dual role during the development and progression of immune-mediated inflammatory diseases. The enhanced TGF-β1 production in tissues induces local fibrogenesis and ultimately causes fulminant organ damage[14].

Our study was performed on 25 children suffering from SLE: (13 of them presented clinically and through laboratory results by active nephritis, 12 were without nephritis). These 2 groups were compared in plasma and urinary levels of TGF-β1 with 20 healthy control subjects of matched age, sex and weight.

The present study showed a highly significant difference between patients with active nephritis and the healthy subjects in plasma TGF-β1 levels (significantly low in group I). While there was no significant difference between patients with silent nephritis and the control group.

In fact, early studies revealed no significant differences of levels of TGF-β1 in serum between healthy control subjects and patients with inactive and active SLE[15].

On the other hand, Sook et al.[16] studied cytokines in 145 SLE patients and 61 control. They found that plasma TGF-β1 was significantly higher in the controls than the patients (p<0.001).

Also, Ohtsuka et al.[17], reported no detectable TGFβ1 production in isolated T cells, but found no difference between TGF-β1 production of monocytes from normal and SLE patients. They found that in SLE and healthy controls, NK cells produced substantially more TGF-β1 than T cells.

Kohut et al.[18] showed that TGF-β1 mRNA production is defective in T and B cells isolated from SLE patients' peripheral blood. In more than half of SLE patients T cells displayed undetectable levels of TGF-β1 mRNA. Nevertheless, they found slightly increased total plasma TGF-β1 levels both in active and in inactive SLE patients. This may seem paradoxical, but NK cells, monocytes and other non lymphoid cells are also potential producers of TGF-β1 .

Our results showed a significant positive correlation between serum TGF-β and C3,while there was a significant negative correlation between it and both serum creatinine, urinary albumin, anti ds-DNA and SLEDAI score.

Hammad et al.[19] agreed with our results and revealed a significantly decreased plasma TGF-β1 levels in children with active SLE when compared to healthy controls. Moreover, plasma active TGF-β1 levels correlated negatively with SLEDAI score. They suggest that decreased plasma TGF-β1 levels with subsequent insufficient exposure of T cells to TGF- β1 might be one of the factors responsible for impaired down regulation of B cells. Inhibition of complement (C3) synthesis by TGF-β1 may be associated with suppression of complement activation locally by blocking renal inflammation in response to high levels of latent TGF-β1[20].

Also, Becker et al.[21] agreed with our results as they found that a lower TGF-ß1 level correlates with disease activity, and severe organ damage in active SLE.

Renal involvement has been reported in approximately two-thirds of children and adolescents with SLE during the course of their disease. However, only 25–50% of unselected patients with lupus were reported to have abnormalities of urine or renal impairment early in their disease[22]. Increased glomerular TGF-β1 was found present and locally produced in samples of adults as well as children with SLE nephritis[23].

Saxena et al.[24] measured tissue concentration of TGF-β1 in kidneys in lupus mice and found that it correlated with the extent of chronic kidney disease. This relationship appears more robust when TGF- β1was measured in the urine of lupus mice. Urinary TGF-β1 levels strongly correlate with chronic kidney damage, particularly with tubulointerstitial disease and glomerulosclerosis.

Also, Sonkar and Singh[25] found that TGF-β1 was significantly high in chronic renal failure (CRF) patients as compared to acute renal failure (ARF), probably it is more associated with interstitial inflammation but it can differentiate CRF from ARF if cut off of 40 ng/ml is taken .

Our results showed a significantly high levels of urinary TGF-β1 in SLE patients (either with active or silent nephritis) relative to the control group. We proved a significant positive correlation between urinary TGF-β1and both anti dsDNA and SLEAI score, while there was a significant negative correlation between urinary TGF-β1 and C3. There was no correlation between urinary TGF-β1 with both serum creatinine and urinary albumin.

This agreed with Honkanen et al.[26] who found a positive correlation between urinary TGF-β1 excretion and interstitial inflammation, denoting that interstitial inflammatory cells may be the source of urinary TGF-β1.

On the contrary, in the study of Haramaki et al.[27] interstitial inflammation was not present in patients with in IgA nephropathy .

In a study performed by Muro et al.[28], urinary TGF-β1 levels correlated with crescent formation, floccular adhesions and mesangial proliferation, but not with the degree of tubulo-interstitial fibrosis.

We were against Hammad et al.[19] who found no significant correlation between urinary TGF-β1 levels and SLEDAI or proteinuria and only plasma active TGF-β1 was negatively correlated with SLEDAI. They also found a positive correlation between urinary TGF- β1 levels and serum anti-ds DNA levels and a negative correlation between urinary TGF-β1 levels and serum C3 levels.

.We conclude that in children with active SLE, lowered plasma TGF- β1 level may be a feature of systemic immune dysfunction, which may have a role in the pathogenesis of renal involvement in those patients. Urinary TGF-β1 plays a role in the clinical presentation of lupus nephritis and has a prognostic value for disease severity. However, further studies are recommended to evaluate the use of plasma and urinary TGF-β1 as alternative non invasive marker for assessment of activity of lupus nephritis

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