Association of Tumor Necrosis Factor-Alpha Gene Polymorphisms With Juvenile Systemic Lupus Erythematosus Nephritis in a Cohort of Egyptian Patients

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Keywords. systemic lupus erythematosus, child, genetic polymorphism, tumor necrosis factor-alpha, lupus nephritis

Introduction. The production of tumor necrosis factor (TNF)-α has been deeply deregulated in systemic lupus erythematosus. We evaluated the association of -863C>A and -1031T>C polymorphisms of the *TNF* gene with susceptibility to and clinical manifestations of juvenile systemic lupus erythematosus.
Materials and Methods. This study was performed on 70 juvenile patients with systemic lupus erythematosus (mean age, 13.0 ± 4.2 years). Ninety age- and sex-matched children served as a controls. All participants were genotyped for the *TNF* -863C>A and -1031T>C polymorphisms by polymerase chain reaction-restriction fragment length polymorphism method. Analysis of serum TNF-α was done by solid-phase sandwich enzyme immunoassay.
Results. The mean serum TNF-α was significantly higher in the SLE patients compared to controls ($P < .001$). Regarding all participants, the mean serum TNF-α was significantly higher in children with -863AA genotype compared to carriers of -863C allele ($P < .001$). The *TNF* -863AA genotype frequencies were significantly higher in the patients group compared with the controls ($P = .005$) and were associated with increased risk for SLE development (odds ratio, 4.05; 95% confidence interval, 1.38 to 13.13; $P = .005$). The -863AA variant was associated with nephritis ($P < .001$) and Raynaud phenomenon ($P = .001$).
Conclusions. The -863A allele of the *TNF* gene can be used as a genetic marker for SLE susceptibility and was associated with high TNF-α production, Raynaud phenomenon, and nephritis in juvenile SLE Egyptian patients.

IJKD 2011;5:392-7
www.ijkd.org

Original Paper

INTRODUCTION

Systemic lupus erythematosus (SLE) is a multisystemic autoimmune disorder, with varying incidence and prevalence between populations. The disease is characterized by autoantibody production, formation of immune complexes, and subsequent tissue inflammation in multiple organs such as the skin, joints, kidneys, and heart.[1] Early renal involvement in childhood SLE is common and serious, and it requires proper evaluation and management.[2] Lupus nephritis is associated with increased mortality and morbidity, particularly among Afro-Caribbean patients. Despite encouraging results for kidney transplantation,

once end-stage renal disease is established, the prognosis is relatively poor and no improvement in preventing its development is achieved.[3]

Systemic lupus erythematosus is caused by a combination of genetic risk factors and environmental events that lead to disease initiation and progression. Given the importance of cytokines in immune system regulation, several circulating cytokine abnormalities have been reported in SLE.[4] Tumor necrosis factor (TNF)-α is a potential pro-inflammatory cytokine that plays an important role in inflammatory and immune responses. Tumor necrosis factor-α stimulates cytokine production, enhancing expression of adhesion molecules and neutrophil activation, and acts as a co-stimulator for T-cell activation and antibody production.[5] Several studies have analyzed the association of TNF-α genetic variants with susceptibility to and outcome of SLE, showing variable results in most cases.[6]

The purpose of the present study was to evaluate the association of -863C>A and -1031T>C polymorphisms of the *TNF* gene with susceptibility to SLE and clinical manifestations of SLE-like nephritis.

MATERIALS AND METHODS

The present study was performed on 70 SLE patients (mean age, 13.0 ± 4.2 years; female-male ratio, 64:6), recruited from the Rheumatology Clinic Department in Cairo University Children Hospital and the Internal Medicine and Rheumatology and Rehabilitation Departments of Cairo University Hospitals. The control group consisted of 90 healthy age- and sex-matched children. Informed consents were taken from the parents of the patients according to the guidelines of the ethical committee of National Research Centre, Egypt.

Full history taking, thorough examination, and laboratory and relevant radiological investigations were performed for all the patients. Diagnosis of SLE was based on the revised criteria of the American College of Rheumatology (ACR) for classification of SLE.[7] Nephritis was estimated clinically in our patients. The total tenderness of joints was counted for patients with arthritis considering tenderness (T) score to be equal to zero, if there is no tenderness, and 1, 2, and 3 if there is mild, moderate, or severe tenderness, respectively. The number of swollen joints was also counted. Systemic lupus erythematosus disease activity was evaluated with the Systemic Lupus Erythematosus Disease Activity Index[8] and the Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index score.[9]

For all of the participants, venous blood samples (5 mL) were collected in plain tubes (2 mL) and ethylenediaminetetraacetic acid tubes (3 mL). Genomic DNA was obtained using the salting out technique.[10] Serum samples and DNA were stored at -20°C until analyzed. Analysis of serum TNF-α was done by solid phase sandwich enzyme immunoassay. The kit was supplied by Quantikine (R&D Systems, Minneapolis, MN, USA). The *TNF* -863C>A and -1031T>C genotypes were determined by polymerase chain reaction-restriction fragment length polymorphism method. The *TNF* -863C>A polymorphism was analyzed using the following primers:

forward: 5′-GGCTCTGAGGAATGGGTTAC-3′
reverse: 5′-CTACATGGCCCTGTCTTCGTTACG-3′

The polymerase chain reaction products were digested with *Tai*I enzyme and visualized on 4% agarose gel stained with ethidium bromide.[11]

The -1031T>C polymorphism was evaluated using the following primers:

forward: 5′-TATGTGATGGACTCACCAGGT-3′
reverse: 5′-CCTCTACATGGCCCTGTCTT-3′

followed by digestion with the restriction enzyme *Bbs*I. The DNA fragments were subjected to electrophoresis in a 2% agarose gel stained with ethidium bromide.[12]

The SPSS software (Statistical Package for the Social Sciences, version 10.0, SPSS Inc, Chicago, Ill, USA) was used for data management and analysis. Quantitative data were presented as mean ± standard deviation. Comparison of quantitative variables between the study groups was done using the Student *t* test. Qualitative data waere expressed as frequency and percentage. Association between qualitative data was done using the chi-square test. Differences with *P* values less than .05 were considered significant. Odds ratio and 95% confidence interval were calculated to estimate the strength of the associations.

RESULTS

Seventy SLE patients were included in this study. They manifested clinically as having Malar rash (39 patients, 57%), Raynaud phenomenon (24

patients, 34%), neuropsychiatric manifestations (19 patients, 27%), nephritis (17 patients, 24 %), arthritis (14 patients, 20%), hematologic abnormalities (16 patients, 23%), and anti-DNA antibody (41 patients, 58%). Ninety healthy children were included as a control group. Laboratory data of the patients are summarized in Table 1.

The mean serum TNF-α was significantly higher in the SLE patients (12.9 ± 4.8 pg/mL) compared to the controls (5.1 ± 3.7 pg/mL; $P < .001$). Among the patients and the controls as a whole, the mean serum TNF-α was significantly higher in children with -863AA genotype (13.2 ± 5.3 pg/mL) compared to carriers of -863C allele (6.4 ± 3.6 pg/mL; $P < .001$). On the other hand, TNF-α levels were comparable between -1031CC (9.1 ± 5.5 pg/mL) and -1031TC and -1031TT variants (8.3 ± 5.1 pg/mL; $P = .16$).

The TNF -863AA genotype frequencies were significantly higher in the patients group compared with the controls ($P = .005$) and were associated with increased risk for SLE development (odds ratio, 4.05; 95% confidence interval, 1.38 to 13.13; $P = .005$). As for TNF -1031T>C polymorphism, no significant differences were found between the patients and the controls (Table 2). With respect to the presence or absence of various clinical phenotypes, the -863AA variant was associated with nephritis ($P < .001$) and Raynaud phenomenon ($P = .001$; Table 3).

Table 1. Laboratory Parameters of Patients With Systemic Lupus Erythematosus

Parameter	Value
Erythrocyte sedimentation rate, mm/h	46.85 ± 29.26
Erythrocyte count, × 10^{12}/L	3.54 ± 0.98
Hemoglobin g/dL	9.84 ± 2.63
Leukocyte count, × 10^{9}/L	6.89 ± 3.86
Platelet count, × 10^{9}/L	353.71 ± 166.48
Asparate aminotransferase, U/L	33.28 ± 18.57
Alanine aminotransferase, U/L	28.55 ± 17.79
Serum albumin, mg/dL	3.47 ± 0.77
Alkaline phosphatase, U/L	151.44 ± 73.31
Serum creatinine, mg/dL	1.02 ± 0.49
Blood urea, mg/dL	38.39 ± 22.54
Serum uric acid, mg/dL	7.20 ± 1.12
Serum calcium, mg/dL	8.65 ± 1.18
Serum triglyceride, mg/dL	216.43 ± 121.98
Serum cholesterol, mg/dL	198.83 ± 79.48
Serum complement 3, g/L	0.61 ± 0.33
Serum complement 4, g/L	0.94 ± 0.72

Table 2. Genotype and Allele Frequencies of Polymorphisms in Tumor Necrosis Factor (TNF)-α Gene in Patients With Systemic Lupus Erythematosus (SLE) and Controls*

Genotypes	Patients With SLE (n = 70)	Controls (n = 90)	*P*
TNF-863			
Genotype			
AA	34 (48.5)	24 (26.6)	.005
AC	28 (40.0)	47 (52.2)	.17
CC	8 (11.4)	19 (21.1)	.02
Alleles			
A	96 (68.6)	95 (52.7)	.001
C	44 (31.4)	85 (47.3)	.001
TNF-1031			
Genotype			
CC	19 (27.1)	20 (22.2)	.63
TC	25 (35.7)	39 (43.3)	.64
TT	26 (37.1)	31 (34.4)	.60
Alleles			
T	77 (55.0)	101 (56.1)	.78
C	63 (45.0)	79 (43.9)	.79

*Values in parentheses are percents.

DISCUSSION

Systemic lupus erythematosus is a disorder of immune regulation resulting in a chronic inflammation that affects many organs. The production of TNF has been found to be deeply deregulated in SLE, suggesting that this regulator of the inflammatory reaction may be involved in the pathogenesis of the disease.[5] In the present

Table 3. Association of Tumor Necrosis Factor (TNF)-α -863C>A Polymorphism With Clinical Manifestation of Systemic Lupus Erythematosus*

Clinical Phenotype	A Allele	C Allele	*P*
Malar rash (n = 39)	54 (56.3)	24 (54.6)	.72
Raynaud phenomenon (n = 24)	39 (40.6)	9 (20.4)	.001
Neuropsychiatric manifestations (n = 19)	25 (26.0)	13 (29.5)	.68
Nephritis (n = 17)	28 (29.0)	6 (13.6)	< .001
Arthritis (n = 14)	18 (18.8)	10 (22.7)	.69
Hematologic abnormalities (n = 16)	23 (23.9)	9 (20.4)	.61
Anti-DNA antibody (n = 41)	53 (55.2)	29 (65.9)	.35

*Values in parentheses are percents.

work, we found that serum TNF-α levels were significantly higher in the SLE patients compared to controls. This is in agreement with previous studies.[13-15] However, in the study of Wais and colleagues, no significant difference was found in plasma TNF-α between SLE patients in clinical remission and controls.[16] On the other hand, some studies demonstrated that TNF-α levels were diminished in SLE.[17,18]

Data concerning the serum levels of TNF-α in patients with SLE are rather controversial. Serum concentrations of TNF-α has been reported as "normal" levels, increasing levels during infectious complications, levels without any clear correlation with the activity of the disease, and levels correlated with acute-phase proteins.[19] Our data showed that the -863AA variant of *TNF* was associated with increased production of TNF, similar results were reported a previous study.[20] On the other hand, Heesen and colleagues[21] showed that the -863A allele was related to decreased serum TNF. However, another study reported that the -863A variant had no association with serum TNF levels.[22] Furthermore, we found no association between -1031T>C polymorphism and TNF production. This is in agreement with results reported by Escobar-Morreale and coworkers and Liu and colleagues.[22,23] However, the -1031C allele was associated with increased synthesis of TNF in other studies.[24,25]

The involvement of TNF-α in the pathogenesis of lupus is a controversial issue. The *TNF* genes have been associated with different forms of cytokine production after in vitro lymphocyte stimulation.[5] Various mechanisms may be involved in the detrimental effect of TNF-α in SLE. Because of its marked pro-inflammatory properties, it is quite plausible that high genetically sustained TNF-α production, acting on an autoimmune-prone genetic background, would result in the maintenance and amplification of an initial immune response to unknown self-peptides.[26] In the current study, we found that -863AA variant of TNF-α gene was associated with increased susceptibility to SLE. Similar findings were reported in studies carried out in Thailand and Taiwan.[20,27] However, our result does not agree with previous reports in Caucasian populations.[28,29] In addition, our data showed that -1031T>C polymorphism was not related to susceptibility to SLE, which is in accordance with the findings of Lin and colleagues.[27] However, Tsuchiya and coworkers[28] suggested that -1031T>C polymorphism was associated with protection against SLE in a United States population. The contradictory results might be attributed to different ethnic origins. Also, Hirankarn and associates[20] suggested that the relation of *TNF* gene polymorphism with SLE susceptibility might be due to linkage disequilibrium with other genes rather than the direct functional effect of *TNF* polymorphism, and Suarez and colleagues[5] reported that the isolated assessment of cytokine genotypes, though of relevance, may not provide a realistic picture of their influence on lupus disease. The actions of cytokines may be profoundly conditioned by the presence of other cytokines, particularly in the case of cytokines which have complex and predominantly opposing roles in the systemic inflammatory responses.

Comparing TNF polymorphisms with respect to the presence or absence of various clinical phenotypes, we found that the -863AA variant was associated with Raynaud phenomenon and nephritis. Association of the -863AA variant with Raynaud phenomenon was also reported in previous studies.[20,29] The pathogenesis of Raynaud phenomenon is believed to be the disturbance of endothelial function.[30,31] Endothelin-1 and inflammatory factors including TNF-α are increased in patients with Raynaud phenomenon. It was proposed that high TNF-α level stimulated the release of endothelin-1 by vascular endothelium, resulting in vasoconstriction, and shifted the balance that controlled basic functions of microcirculation. Therefore, it is possible that SLE patients with a genetic background of high TNF-α production should have a greater risk of Raynaud phenomenon.[32]

As for nephritis, our results are consistent with other studies which showed that both genes related to TNF-α and human leukocyte antgen-DR2 may play a role in SLE susceptibility. Also, lupus nephritis was strongly associated with the *TNF* gene.[33] Furthermore, a recent study by Santos and associates showed that lupus nephritis was significantly more prevalent among SLE patients possessing the *TNF* -308A allele (odds ratio, 2.84; 95% confidence interval, 1.14 to 7.03; $P = .02$). The occurrence of nephritis was also higher in the *LTA* 252G allele carriers (odds ratio, 2.90;

95% confidence interval, 1.12 to 7.54; $P = .02$). These polymorphisms appear to associate with the risk of renal lupus and distinct immunological features.[34] Nonetheless, our results disagree with those reported by Lin and colleagues[27] who found no association between -863C>A polymorphism and lupus nephritis.

It has been reported that TNF-α serum and urine concentrations are significantly increased in patients with lupus nephritis compared with normal controls, which suggests that TNF-α has a role in the pathogenesis of nephritis[13,35]; however, *TNF* genotyping was not performed in these studies.

The association of -863A allele with lupus nephritis is consistent with our finding of increased TNF-α levels in association with this variant and suggests that the -863A allele may be involved in risk for nephritis, in part, through its higher promoter activity of TNF-α production. These results may help in suspecting cases with nephritis, which need meticulous follow-up trying to avoid end-stage renal diseases. Roozbeh and coworkers suggested no difference in outcomes of first kidney transplantation in patients with SLE and non-SLE patients apart from longer hospital stay in the SLE group.[36] The prognosis of lupus nephritis remains unsatisfactory. Besides exploring more effective but less toxic treatment modalities that will further improve the remission rate, early detection and treatment of renal activity may spare patients from intensive immunosuppressive therapies and reduce kidney damage.[37]

CONCLUSIONS

The -863A allele of the *TNF* gene can be used as a genetic marker for SLE susceptibility. This polymorphism was associated with high TNF-α production, Raynaud phenomenon, and nephritis in SLE patients. The *TNF* -1031T>C polymorphism was not related to susceptibility to or clinical phenotypes of SLE in Egyptian children. Thus, it is recommended that genotyping for *TNF* -863C>A polymorphism be performed in SLE patients, as it associated with important manifestations of the disease, such as nephritis. Also, genotyping may be used in high-risk groups for prediction of the disease before any renal manifestations.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Danchenko N, Satia JA, Anthony MS. Epidemiology of systemic lupus erythematosus: a comparison of worldwide disease burden. Lupus. 2006;15:308-18.
2. Mahmoud SS, Bazaraa HM, Lotfy HM, Abd-El-Aziz DM. Renal involvement in childhood-onset systemic lupus erythematosus in Egypt. Rheumatol Int. 2010.
3. Croca SC, Rodrigues T, Isenberg DA. Assessment of a lupus nephritis cohort over a 30-year period. Rheumatology (Oxford). 2011;50:1424-30.
4. Tsao BP. Update on human systemic lupus erythematosus genetics. Curr Opin Rheumatol. 2004;16:513-21.
5. Suarez A, Lopez P, Mozo L, Gutierrez C. Differential effect of IL10 and TNF{alpha} genotypes on determining susceptibility to discoid and systemic lupus erythematosus. Ann Rheum Dis. 2005;64:1605-10.
6. Lopez P, Gomez J, Prado C, Gutierrez C, Suarez A. Influence of functional interleukin 10/tumor necrosis factor-alpha polymorphisms on interferon-alpha, IL-10, and regulatory T cell population in patients with systemic lupus erythematosus receiving antimalarial treatment. J Rheumatol. 2008;35:1559-66.
7. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum. 1997;40:1725.
8. Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang CH. Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. Arthritis Rheum. 1992;35:630-40.
9. Gladman D, Ginzler E, Goldsmith C, et al. The development and initial validation of the Systemic Lupus International Collaborating Clinics/American College of Rheumatology damage index for systemic lupus erythematosus. Arthritis Rheum. 1996;39:363-9.
10. Noguera NI, Tallano CE, Bragos IM, Milani AC. Modified salting-out method for DNA isolation from newborn cord blood nucleated cells. J Clin Lab Anal. 2000;14:280-3.
11. Skoog T, van't Hooft FM, Kallin B, et al. A common functional polymorphism (C-->A substitution at position -863) in the promoter region of the tumour necrosis factor-alpha (TNF-alpha) gene associated with reduced circulating levels of TNF-alpha. Hum Mol Genet. 1999;8:1443-9.
12. van Heel DA, Udalova IA, De Silva AP, et al. Inflammatory bowel disease is associated with a TNF polymorphism that affects an interaction between the OCT1 and NF(-kappa)B transcription factors. Hum Mol Genet. 2002;11:1281-9.
13. Mahmoud RA, El-Gendi HI, Ahmed HH. Serum neopterin, tumor necrosis factor-alpha and soluble tumor necrosis factor receptor II (p75) levels and disease activity in Egyptian female patients with systemic lupus erythematosus. Clin Biochem. 2005;38:134-41.
14. Aringer M, Zimmermann C, Graninger WB, et al. TNF-a is an essential mediator in lupus nephritis. Arthritis Rheum. 2002; 46:3418-9.
15. Swadzba J, Iwaniec T, Musial J. Increased level of tumor necrosis factor-alpha in patients with antiphospholipid syndrome: marker not only of inflammation but also of the

prothrombotic state. Rheumatol Int. 2011;31:307-13.

16. Wais T, Fierz W, Stoll T, Villiger PM. Subclinical disease activity in systemic lupus erythematosus: immunoinflammatory markers do not normalize in clinical remission. J Rheumatol. 2003;30:2133-9.
17. Mageed RA, Isenberg DA. Tumour necrosis factor alpha in systemic lupus erythematosus and anti-DNA autoantibody production. Lupus. 2002;11:850-5.
18. Gomez D, Correa PA, Gomez LM, Cadena J, Molina JF, Anaya JM. Th1/Th2 cytokines in patients with systemic lupus erythematosus: is tumor necrosis factor alpha protective? Semin Arthritis Rheum. 2004;33:404-13.
19. Dean GS, Tyrrell-Price J, Crawley E, Isenberg DA. Cytokines and systemic lupus erythematosus. Ann Rheum Dis. 2000;59:243-51.
20. Hirankarn N, Avihingsanon Y, Wongpiyabovorn J. Genetic susceptibility to SLE is associated with TNF-alpha gene polymorphism -863, but not -308 and -238, in Thai population. Int J Immunogenet. 2007;34:425-30.
21. Heesen M, Kunz D, Wessiepe M, van der Poll T, Zwinderman AH, Blomeke B. Rapid genotyping for tumor necrosis factor-alpha (TNF-alpha)-863C/A promoter polymorphism that determines TNF-alpha response. Clin Chem. 2004;50:226-8.
22. Escobar-Morreale HF, Calvo RM, Sancho J, San Millan JL. TNF-alpha and hyperandrogenism: a clinical, biochemical, and molecular genetic study. J Clin Endocrinol Metab. 2001;86:3761-7.
23. Liu Y, Jin W, Lu L, Chen QJ, Shen WF. [Association between the -1031T/C polymorphism in tumor necrosis factor-alpha gene and unstable angina]. Zhonghua Yi Xue Yi Chuan Xue Za Zhi. 2009;26:571-4. Chinese.
24. Sohail M, Kaul A, Bali P, et al. Alleles -308A and -1031C in the TNF-alpha gene promoter do not increase the risk but associated with circulating levels of TNF-alpha and clinical features of vivax malaria in Indian patients. Mol Immunol. 2008;45:1682-92.
25. Inoue N, Watanabe M, Nanba T, Wada M, Akamizu T, Iwatani Y. Involvement of functional polymorphisms in the TNFA gene in the pathogenesis of autoimmune thyroid diseases and production of anti-thyrotropin receptor antibody. Clin Exp Immunol. 2009;156:199-204.
26. Korner H, Cretney E, Wilhelm P, et al. Tumor necrosis factor sustains the generalized lymphoproliferative disorder (gld) phenotype. J Exp Med. 2000;191:89-96.
27. Lin YJ, Chen RH, Wan L, et al. Association of TNF-alpha gene polymorphisms with systemic lupus erythematosus in Taiwanese patients. Lupus. 2009;18:974-9.
28. Tsuchiya N, Kawasaki A, Tsao BP, Komata T, Grossman JM, Tokunaga K. Analysis of the association of HLA-DRB1, TNFalpha promoter and TNFR2 (TNFRSF1B) polymorphisms with SLE using transmission disequilibrium test. Genes Immun. 2001;2:317-22.
29. McHugh NJ, Owen P, Cox B, Dunphy J, Welsh K. MHC class II, tumour necrosis factor alpha, and lymphotoxin alpha gene haplotype associations with serological subsets of systemic lupus erythematosus. Ann Rheum Dis. 2006;65:488-94.
30. Hajeer AH, Worthington J, Davies EJ, Hillarby MC, Poulton K, Ollier WE. TNF microsatellite a2, b3 and d2 alleles are associated with systemic lupus erythematosus. Tissue Antigens. 1997;49:222-7.
31. Herrick AL. Pathogenesis of Raynaud's phenomenon. Rheumatology (Oxford). 2005;44:587-96.
32. Rychlik-Golema W, Mastej K, Adamiec R. The role of endothelin-1 and selected cytokines in the pathogenesis of Raynaud's phenomenon associated with systemic connective tissue diseases. Int Angiol. 2006;25:221-7.
33. Wang M, Dong Y, Huang S. [Study on the association between tumor necrosis factor alpha gene polymorphism and systemic lupus erythematosus]. Zhonghua Nei Ke Za Zhi. 1999;38:393-6. Chinese.
34. Santos MJ, Carmona-Fernandes D, Caetano-Lopes J, et al. TNF promoter -308 G>A and LTA 252 A>G polymorphisms in Portuguese patients with systemic lupus erythematosus. Rheumatol Int. 2011.
35. Tesar V, Jirsa M, Jr., Zima T, et al. Soluble cytokine receptors in renal vasculitis and lupus nephritis. Med Sci Monit. 2002;8:BR24-9.
36. Roozbeh J, Eshraghian A, Raeesjalali G, et al. Outcomes of kidney transplantation in patients with systemic lupus erythematosus: a single-center study. Iran J Kidney Dis. 2011;5:53-6.
37. Mok CC. Biomarkers for lupus nephritis: a critical appraisal. J Biomed Biotechnol. 2010;10:1155-70.

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Received January 2011
Revised June 2011
Accepted June 2011