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The Expression of Migration Inhibitory Factor mRNA in Peripheral Blood of Children with Systemic Lupus Erythematosus

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Abstract

Aim: This study was aiming at evaluation of the importance and the change in the level of macrophage migration inhibitory factor (MIF) in serum studied by ELISA and in peripheral blood mononuclear cell (PMBC) through the expression of mRNA of MIF studied by reverse PCR and its correlation with disease activity index in patients with systemic lupus erythematosus (SLE). Also, the relationship of MIF with the treatment by corticosteroids and renal involvement was taken into consideration.

Material and methods: For this purpose, twenty lupus patients, regularly attending the Pediatric Allergy and Immunology Clinic, Children Hospital, Ain Shams University and fulfilling the American Rheumatism Association Revised Criteria for diagnosis of SLE, were studied in comparison to thirty-five healthy subjects as normal controls. According to the presence or absence of clinical renal involvement, lupus patients were divided into two groups: Group Ia: included 10 patients with clinical renal involvement and Group Ib: comprised 10 patients without clinical renal involvement. Full history taking and clinical examination were done and the SLEDAI was assessed. Also, the following laboratory investigations were performed ESR, routine microscopic urine analysis, 24 hours urine proteins, serum ANA, C3, serum anti-DNA, serum creatinine, serum MIF by ELISA and MIF gene expression through the mRNA in PBMC by rPCR.

Results: The results of the present study showed that the MIF in serum and in PBMC were significantly higher among lupus patients as compared to normal controls. In addition, both were significantly higher among lupus patients with clinically evident nephritis than those without. Serum MIF and MIF gene expression were both significantly higher among lupus patients who suffered from symptoms suggestive of lupus nephritis as edema, hematuria, hypertension and positive anti DNA levels than other patients who did not experience these symptoms. In our study, different correlation analysis were done with serum MIF and MIF gene expression and proved to be significantly positive with SLEDAI score, ESR, cumulative dose of steroids, serum creatinine, 24 hour urinary protein and duration of illness in all lupus patients.

Conclusions: In conclusion, MIF both in the serum and MIF gene expression by reverse PCR in macrophages were significantly higher in all lupus patients in comparison to healthy controls being significantly higher in those lupus patients with clinically evident nephritis than those without. Also MIF had a significant positive correlation to SLEDAI score and ESR indicating its correlation with disease activity.

Keywords: Systemic lupus erythematosus; Peripheral blood; Children mRNA

Abbreviations

ANA: Anti-Nuclear Antibody; Anti MIF: Anti Macrophage Migration Inhibitory Factor; Anti-BM Antibodies: Anti Basement Membrane Antibodies; Anti-GBM GN: Anti Glomerular Basement Membrane Glomerulonephritis; Anti-Sm: Anti-Smith; Anti-βGP1: Anti Beta 2 Glycoprotein 1; APS: Antiphospholipids Syndrome; ARPA: Antiribosomal P Antibody; AVN: Avascular Necrosis; C cell: Collagen cell; C1q: Complement 1q; C3: Complement 3; C3d: Complement 3d; C4: Complement 4; C4d: Complement 4d; CD4+: Cluster of Differentiation 4+; CD40L: Cluster of Differentiation 40 Lymphocyte; CD8+: Cluster of Differentiation 8+; cDNA: Complementary DNA; CII: Type II Collagen; CNS: Central Nervous System; CPK: Creatine Phosphokinase; CPT: Cell Preparation Tube; Creatinine Cl: Creatinine clearance; CRF: Corticotrophin Releasing Factor; Cumm. Dose: Commulative dose of steroid; DNA: Deoxy Ribonucleic Acid; ds-DNA: Double stranded Deoxyribonucleic Acid; EEG: Electroencephalogram; ELISA: Enzyme Linked Immunosorbant Assay; ESR: Erythrocyte Sedimentation Rate; FC: Fragment Constant; FNA: Fluorescent Antinuclear Antibody; GFR: Glomerular Filtration Rate; GN: Glomerulonephritis; ICAM-1: Intercellular Cell Adhesion Molecule; IgA: Immunoglobulin A; IgG2a: Immunoglobulin G2a; IL: Interleukin Nephritis; iNOS: Inducible Nitric Oxide Synthase; IQR: Interquartile Range; MIF: Macrophage Migration Inhibitory Factor; MIF G expression: Gene expression by revere PCR; /mo: Per month; MR: Magnetic Resonance; mRNA: Messenger RNA (Ribonucleic acid); n:

Number; NK Cell: Natural Killing Cell; NO: Nitric Oxide; NSAIDs: Non-Steroidal Anti-Inflammatory Drugs; OD: Optical Density; P: Plasma creatinine in mg/ml; PBMC: Peripheral Blood Mononuclear Cell; RA: Rheumatoid Arthritis; RNA: Ribonucleic Acid; rPCR: Reverse Polymerase Chain Reaction; S. creatinine: Serum creatinine; ± SD: Standard Deviation; Serum MIF: Serum concentration of macrophage migration inhibitory factor detected by ELISA; SLE: Systemic Lupus Erythematosus; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index; T1: Thoracic 1; Th1: T helper 1; Th2: T helper 2: Thin section; CT: Thin section Computerized Tomography; TNF: Tumor Necrosis Factor; TNF-α: Tumor Necrosis Factor Alpha; U: Urine creatinine in mg/ml; US: Ultrasound; V: Urine volume in ml/min; VCAM-1: Vascular Cell Adhesion Molecule; WHO: World Health Organization

Introduction

Systemic lupus erythematosus (SLE) is a prototype systemic autoimmune disease characterized by autoantibody production. Although the outcome for SLE patients has improved in recent years, SLE continues to profoundly affect health status and the likelihood of disability and premature death. How to ameliorate the reduced quality of life and increase the survival rate in these patients remains a major challenge for rheumatologists. Glucocorticoid (GC) therapy plays an important role in the treatment of SLE, but irreversible side effects limit its use at high doses or over the long term while some patients with highly active SLE do not respond to GC therapy. There is thus a need to investigate the underlying mechanisms behind steroid resistance. Macrophage migration inhibitory factor (MIF) arouses our interest because of its unique relationship with GCs [1].

Although initially described as a product of activated T cells [2], MIF is now known to be produced by a variety of cell types, predominantly by macrophages [3]. Increasing evidence indicates that MIF is a broad-spectrum proinflammatory cytokine that can increase the expression of a number of inflammatory molecules, including TNFα, IL-6, IL-1β, IL-2, IL-8, and IFNγ [4]. In contrast to other proinflammatory cytokines that are generally suppressed by GCs, MIF expression and secretion are induced by low physiological concentrations of GCs [4]. Evidence of upregulation of MIF by endogenous GC has been reported in rat adjuvant-induced arthritis [5]. Regulation of serum MIF by exogenous GC in humans has also been demonstrated in patients with SLE, where it has been observed that serum MIF was influenced by the GC dose even after adjusting for disease activity variables [6]. Despite being induced by GC, MIF exhibits GC-antagonistic effects *in vitro* and *in vivo*. In murine antigen-induced arthritis, GC inhibition of histological severity of disease is reversed by exogenous MIF [7]. Consistent with this finding, Leech and colleagues reported that increased joint inflammation and lethality can be overridden by the neutralization of MIF in the absence of GC in rat adjuvant-induced arthritis [8]. The reciprocal relationship between MIF and GCs in the control of the inflammatory response was also reported in human subjects [9,10]. Several studies to date have revealed that deficiency of MIF, either through genetic deletion or by the anti-sense oligonucleotide, leads to a left-shift in the dose response to GC of macrophage TNF production, which represents the fact that MIF does indeed directly regulate GC sensitivity [5,11].

The mechanism by which MIF counter-regulates the anti-inflammatory actions of GC has not been fully elucidated. However, there are several pathways through which MIF and GCs may interact with each other. One such pathway involves the activation of the transcriptional factor NF-κB. In its inactive state, NF-κB is sequestered in the cytoplasm by the inhibitory protein IκB, and the phosphorylation of the latter results in its ubiquination and degradation by the proteasome, unmasking a nuclear localization signal on NF-κB. In the nucleus, NF-κB binds to DNA sequences called NF-κB elements and is responsible for the transcription of cytokines, chemokines and cell adhesion molecules [12]. The IκB protein binds to activated NF-κB in the cell nucleus, causing dissociation of the latter from the IκB binding sites of the target genes and its subsequent relocation in the cytoplasm [13,14]. GCs prevent NF-κB activation by increasing the expression of IκBα [15,16], which keeps the NF-κB/IκB complex in cytosol and thus prevents the synthesis of inflammatory mediators.

Some studies have shown that MIF plays an important role in the pathogenesis of SLE particularly in lupus nephritis and suggest that MIF blockade may be promising as a new strategy for the treatment of SLE. Also detection of MIF levels may be a useful parameter for monitoring disease activity in patients with SLE [17].

The aim of this work is` to investigate the changes in macrophage migration inhibitory factor mRNA expression in peripheral blood mononuclear cells in relation to disease activity and renal affection in patients with systemic lupus erythematosus.

Subjects and Methods

This study was conducted on twenty patients with SLE, regularly attending the Pediatric Allergy and Immunology Clinic, Children's Hospital, Ain Shams University. All patients were fulfilling the American Rheumatism Association Revised Criteria for diagnosis of SLE [18].They were 16 females and 4 males. Their ages ranged between 10 and 18 years (mean ± SD=15.45 ± 2.50 years, interquartile range: =13.75-18 years). Patients were studied in comparison to 35 healthy children.

Lupus patients were categorized into two groups according to the renal involvement as follows:

Group Ia (patients with clinical renal involvement)

It included 10 patients (6 females and 4 males). Their ages ranged between 11 and 18 years (mean ± SD=15.30 ± 2.21 years, interquartile range=14.25-16.75 years). These patients had renal involvement in the form of persistent proteinuria (more than 0.5 gm/day or more than 3+ by dipstick test) and/or cellular casts in urine (red blood cell, haemoglobin, granular, tubular or mixed) [19].

Group Ib (patients without clinical renal involvement)

It included 10 female patients. Their ages ranged between 10 and 18 years (mean ± SD=15.60 ± 2.88 years, interquartile range=13.5-18 years). These girls had no clinical evidence of renal involvement based on normal results of urine analysis, normal serum creatinine and normal creatinine clearance.

Control group (group II)

Results of the previous two groups were compared with 35 age and sex matched control children.

It comprised (23 females and 12 males) age and sex matched apparently healthy subjects with no history of renal problems and with

normal results of urine analysis. Their ages ranged between 10 and 18 years (mean ± SD=15.77 ± 2.07 years, interquartile range=15-17 years).

Methods

Subjects in this study underwent the following:

1. Full history taking laying stress on the age, duration of the disease, urinary symptoms, SLE manifestations (e.g. joint pains, rash, cutaneous photosensitivity, Raynaud's phenomenon, CNS symptoms including seizures), symptoms of hypertension as vomiting, headache, blurred vision and the type of therapy received by the patient.
2. Thorough clinical examination was performed to each subject including: anthropometric measures weight, height and surface area by using Normogram for estimation of surface area, blood pressure measurement, skin rash distribution, joint affection, chest and heart examination, abdominal examination for hepatospleenomegaly and CNS examination especially the level of consciousness, motor and sensory systems.
3. All systems included in the SLEDAI data collection sheet [20] were assessed clinically to calculate the total SLE activity score.

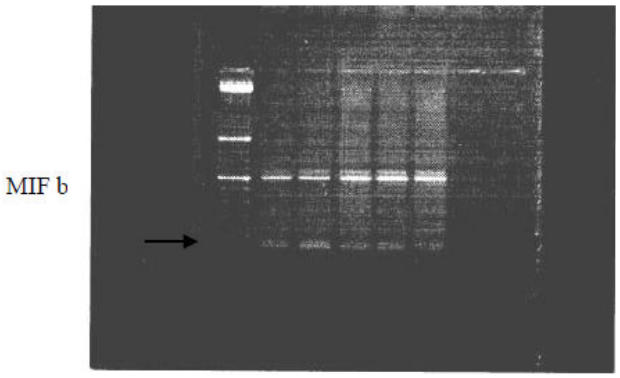

Figure 1: Electrophoretic separation of PCR product on 2% agarose gel. Lane 1 is for the DNA molecular weight marker lane. Lane 2 is positive control. Lane 7-8 are the negative control. Lane 3, 4, 5, 6 we considered positive samples.

RNA extraction

RNA extraction and the mononuclear cells pellet was separated and resuspended then used for RNA extraction. This was carried out using Purescript Total RNA Isolation Kit which was supplied by Gentra, Cat. No. 5500A, USA.

The yield of extracted RNA was determined spectro-photometrically at 260 nm where 1 absorbance unit (A260) equals 40 µg of single standard RNA/ml.

Quantification of macrophage migration inhibitory factor (MIF) mRNA (Figure 1)

A. Amplification step (RT-PCR): It includes,

1. Complementary DNA (cDNA) synthesis.
2. Amplification of MIF cDNA by nested PCR using the following primer [21]

Primers include:

- MIF: for nested PCR reaction according to Matsui et al. [20] supplied by Gulf Tech SA.
- The outer P1, P2:

P15′-CTC TCC GAG CTC ACC CAG CAG-3′ forward (58/78)

P25′-CGC GTT CAT GTC GTA AGT TCA-3′ reverse (292/312)

- The inner primers P3, P4:

P35′-CGT TCT GGC GGC ACC ACC AT-3′ forward (936/935)

P45′-CGA ACT AAG TCC ATA AGT CCG-3′ reverse (1170/1189)

1. Amplification of β-globulin as an external control.

B. Detection of PCR product by gel electrophoresis (according to Sambrook et al. [22]):

Interpretation of results:

The gel was examined under ultraviolet light. Photographs were taken using Polaroid camera:

1. Marker gave different bands.
2. The negative control was examined to exclude any source of contamination.

C. Quantification of MIF mRNA by gel documentation system:

The quantitation step was done using Gel documentation system (Gel Pro-Analzyer Version 3.1) Software Media Cybermetica USA (a software system used for end point quantitation of nucleic acid samples).

The photographed gel was analyzed using the gel document system (Gel Pro Version 0.3) to get the maximum optical density of the bands of the PCR products. A plot was constructed for the input copy number of the standards in relation to the optical density (OD) of the product bands. Using the constructed curve, the input copy number of the sample was anticipated from the OD of its product.

Statistical methods

Data were analyzed using statistical software SPSS for windows, quantitative variables were presented as mean ± standard deviation (M ± SD) Spearman's correlation test was used for correlation within the studied groups, comparison of mean values of various variables between studied groups was done using student t-test for normally distributed values and Mann-Whitney u-test for non-parametric data, for all tests used a p-value <0.05 was considered significant.

Results

The results of data analysis are presented in Tables 1-5.

Table 1 shows the basic clinical and laboratory results of patients with SLE (16 females and 4 males). Their ages ranged between 10 and 18 years (mean ± SD=15.45 ± 2.50 years), median (IQR)=15.5 (4.25) years. Duration of the disease ranged between 1 and 10 years (mean ± SD=3.95 ± 2.42 years), median (IQR)=3.5 (3) years. Their SLEDAI scores ranged between 12 and 60 (mean ± SD=36.20 ± 17.96), median (IQR)=33 (34.5). All the SLE patients were seropositive for ANA.

Patients with clinical renal involvement (group Ia) their serum C3 levels ranged between 29-134 and (mean ± SD=70.00 ± 37.55), median (IQR)=57 (59.5). While patients without clinical renal involvement (group IIb) had serum C3 levels ranging between 45 and 107 (mean ± SD=75.00 ± 19.94), median (IQR)=72 (25.5).

Also, patients with clinical renal involvement (group Ia) had serum MIF levels ranging between 19.9-24.8 ng/ml and (mean ± SD=22.67 ± 1.64 ng/ml), median (IQR)=22.6 (2.05) ng/ml. While in patients without clinical renal involvement (group IIb), serum MIF levels ranged between 11.3 and 13.6 ng/ml (mean ± SD=12.25 ± 0.70 ng/ml), median (IQR)=12.15 (0.625) ng/ml.

Among patients with clinical renal involvement, MIF gene expression levels ranged between 0.20 and 0.27 ng/ml (mean ± SD=0.24 ± 0.02 ng/ml), median (IQR =0.24 (0.039) ng/ml. While patients without clinical renal involvement the MIF gene expression levels ranged between 0.12 and 0.17 ng/ml (mean ± SD=0.15 ± 0.02 ng/ml), median (IQR)=0.15 (0.03) ng/ml.

Serum MIF and MIF G expression levels in relation to the presence or absence of some indicators of lupus nephritis (hematuria, edema, hypertension and anti-DNA) (Tables 1-5)

Serum level of macrophage migration inhibitory factor by ELISA was found to have significantly higher values in patients with either (hematuria, edema, hypertension and anti DNA +ve) than those patients without any of these symptoms.

Macrophage migration inhibitory factor gene expression was found to have significantly higher values in patients with either (hematuria, edema, hypertension and anti DNA +ve) than those patients without any of these symptoms.

Age (in years)	
Range	10-18
Mean ± SD	15.46 ± 2.50
Median (interquartile range)	15.5 (4.25)
Sex	
Male	4
Female	16
Duration of the disease (in years)	
Range	01-10
Mean ± SD	3.95 ± 2.42
Median (interquartile range)	3.5 (2)
SLE-DAI	
Range	12-60
Mean ± SD	36.20 ± 17.96
Median (interquartile range)	33 (34.5)
ANA	
Positive	20
Negative	0
Serum C3	
Patients with clinical renal involvement	
Range	29-134
Mean ± SD	70.00 ± 37.55
Median (IQR)	57 (59.5)
Patients without clinical renal involvement	
Range	45-107
mean ± SD	75.00 ± 19.94

Median (IQR)	72 (25.5)
Serum MIF ng/ml	
Patients with clinical renal involvement	
Range ng/ml	19.9-24.8
Mean ± SD	22.67 ± 1.46
Median (IQR)	22.6 (2.05)
Patients without clinical involvement	
Range ng/ml	11.3-13.6
Mean ± SD	12.25 ± 0.70
Median (IQR)	12.15 (0.63)
MIF gene expression	
Patients with clinical renal involvement	
Range	0.20-0.27
Mean ± SD	0.24 ± 0.02
Median (IQR)	0.24 (0.04)
Patients without clinical renal involvement	
Range	0.12-0.17
Mean ± SD	0.15 ± 0.02
Median (IQR)	0.15 (0.03)
(IQR)=Interquartile range	

Table 1: Basic clinical and laboratory data of patients with SLE.

		Lupus patients with hematuria	Lupus patients without hematuria	ZP
Serum	Mean ± SD	20.52 ± 4.64	14.40 ± 4.57	2.96
MIF (ng/ml)	Median (IQ)	22.1 (3.8)	12.5 (1.38)	0.0083
MIF G expression (ng/ml)	Mean ± SD	0.22 ± 0.05	0.17 ± 0.04	2.52
	Median (IQ)	0.22 (0.05)	1.56 (0.03)	0.0212

Table 2: Serum MIF and MIF G expression levels in relation to the presence or absence of hematuria.

		Lupus patients with edema	Lupus patients without edema	ZP
Serum MIF (ng/ml)	Mean ± SD	22.43 ± 1.54	13.39 ± 3.84	7.13
	Median (IQ)	22.5 (1.4)	12.2 (1)	<0.0001
MIF G expression (ng/ml)	Mean ± SD	0.23 ± 0.02	0.16 ± 0.04	5.44
	Median (IQ)	0.23 (0.03)	0.15 (0.03)	<0.0001

Table 3: Serum MIF and MIF G expression levels in relation to the presence or absence of edema.

		Lupus patients with hypertension	Lupus patients without hypertension	ZP
Serum MIF (ng/ml)	Mean ± SD	21.58 ± 3.18	13.34 ± 3.96	5.13
	Median (IQ)	22.4 (2.3)	12.15 (0.63)	<0.0001
MIF G expression (ng/ml)	Mean ± SD	0.23 ± 0.03	0.15 ± 0.04	5.03
	Median (IQ)	0.23 (0.04)	0.15 (0.03)	<0.0001

Table 4: Serum MIF and MIF G expression levels in relation to the presence or absence of hypertension.

		Lupus patients with anti-DNA +ve	Lupus patients with anti-DNA -ve	ZP
Serum MIF (ng/ml)	Mean ± SD	19.96 ± 4.68	13.71 ± 4.52	2.96
	Median (IQ)	22.1 (4.53)	12.25 (0.93)	0.0083
MIF G expression (ng/ml)	Mean ± SD	0.22 ± 0.04	0.16 ± 0.05	2.92
	Median (IQ)	0.22 (0.05)	0.15 (0.04)	0.00091
IQR=interquartile range, P>0.05=non-significant, P<0.05=significant Macrophage migration inhibitory factor by ELISA=serum MIF				

Table 5: Serum MIF and MIF G expression levels in relation to the presence or absence of anti-DNA.

Correlation analysis

Serum MIF level and gene expression had positive significant correlation with values of SLEDAI (Figure 2), duration of illness by years of SLE patients (Figure 3), values of cumulative dose of steroids (Figure 4), ESR recorded values (Figure 5), serum creatinine levels (Figure 6) and recorded values of 24 hour urinary proteins (Figure 7).

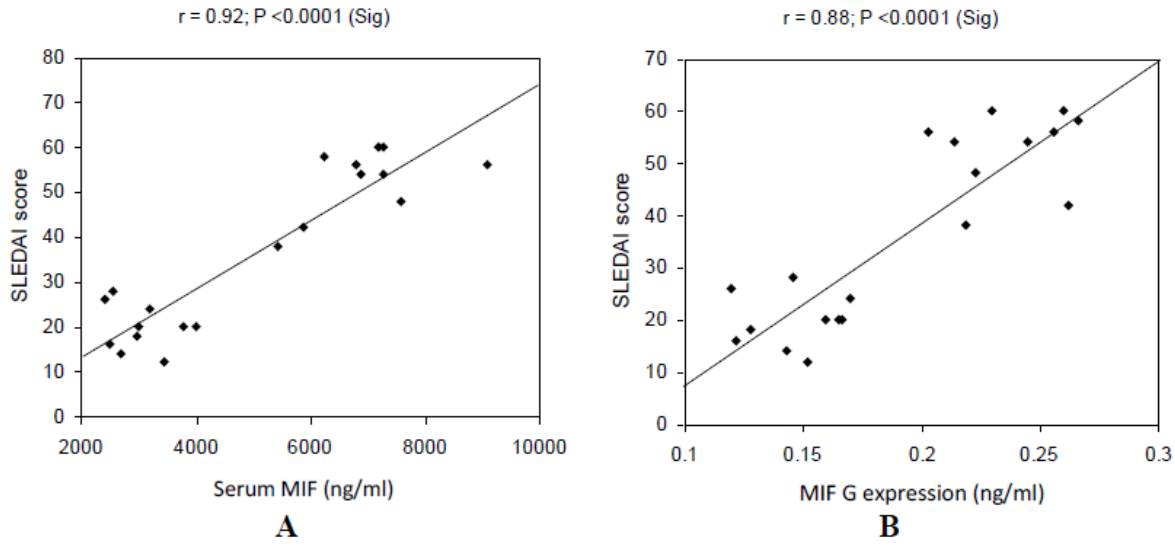

Figure 2: Correlation between the (serum MIF and MIF G expression) and SLEDAI among all lupus patients.

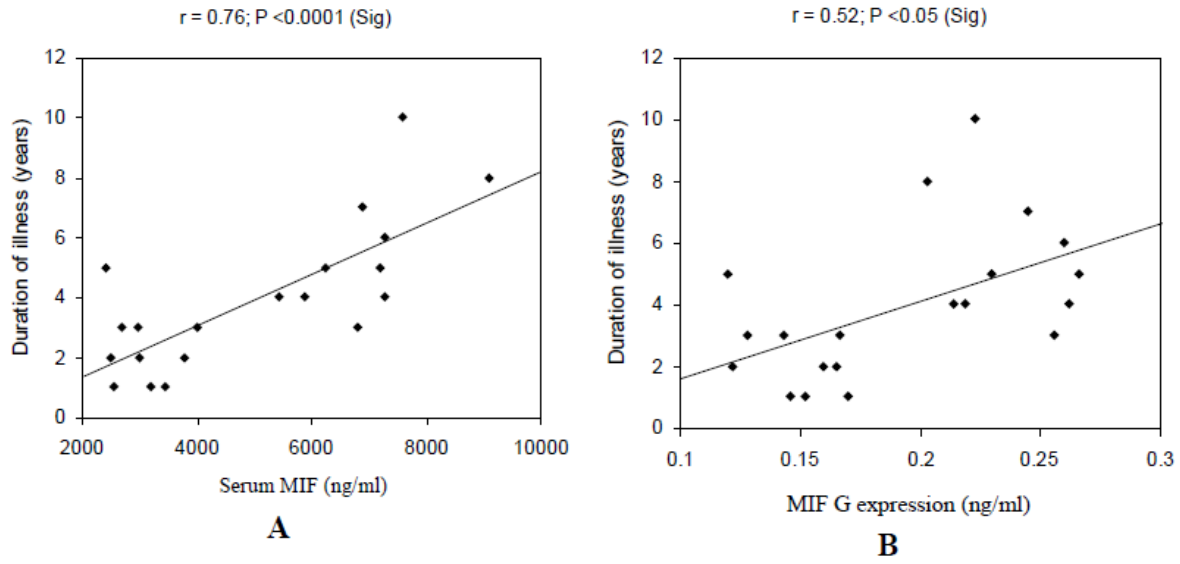

Figure 3: Correlation between the (serum MIF and MIF G expression) and duration of illness among all lupus patients.

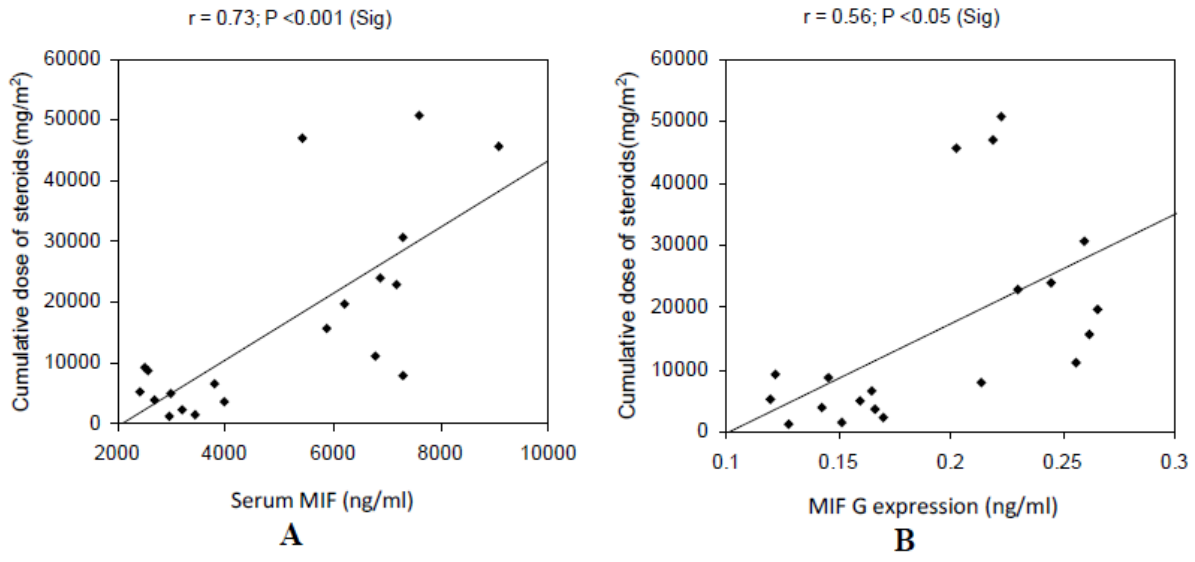

Figure 4: Correlation between the (serum MIF and MIF G expression) and cumulative dose of steroid among all lupus patients.

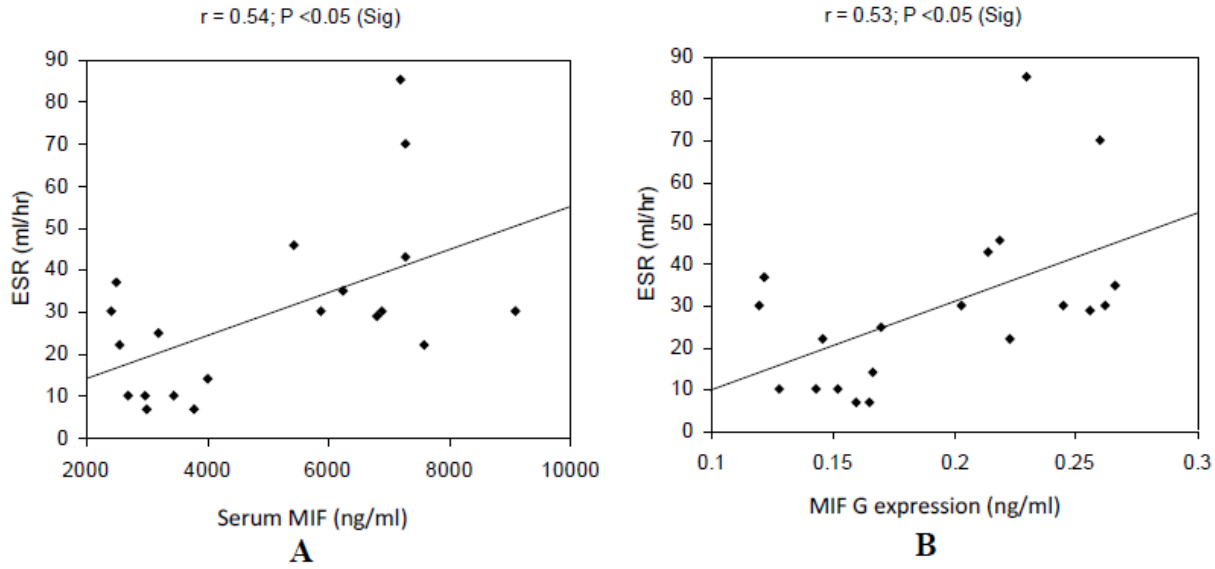

Figure 5: Correlation between the (serum MIF and MIF G expression) and ESR among all lupus patients.

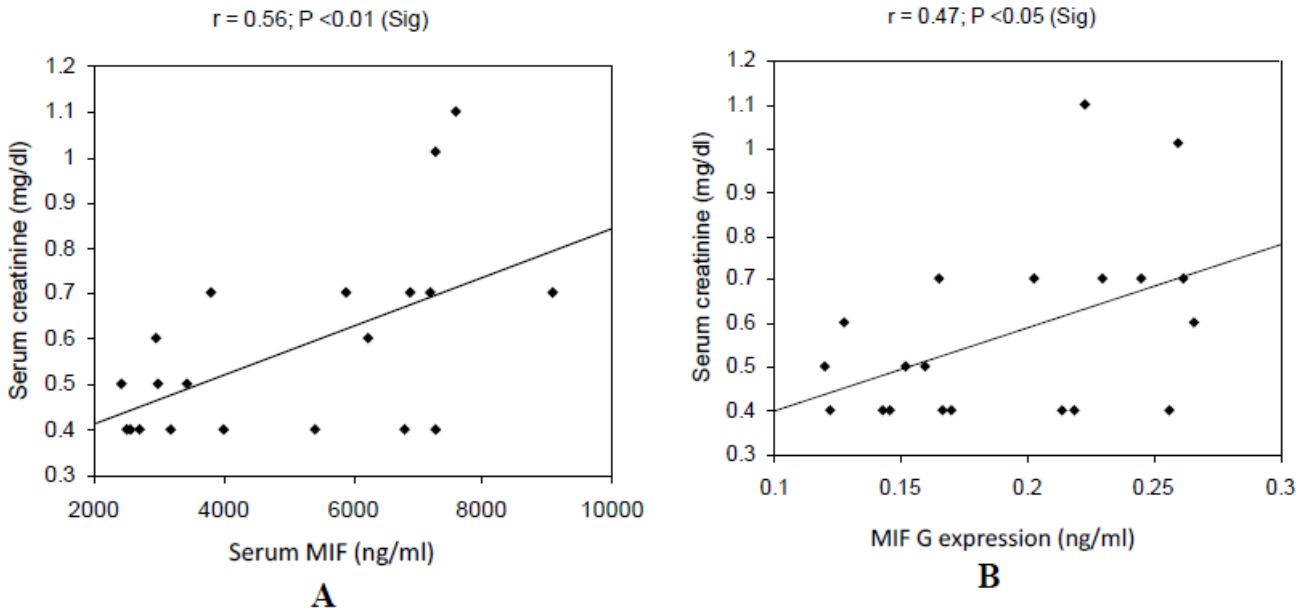

Figure 6: Correlation between the (serum MIF and MIF G expression) and serum creatinine among all lupus patients.

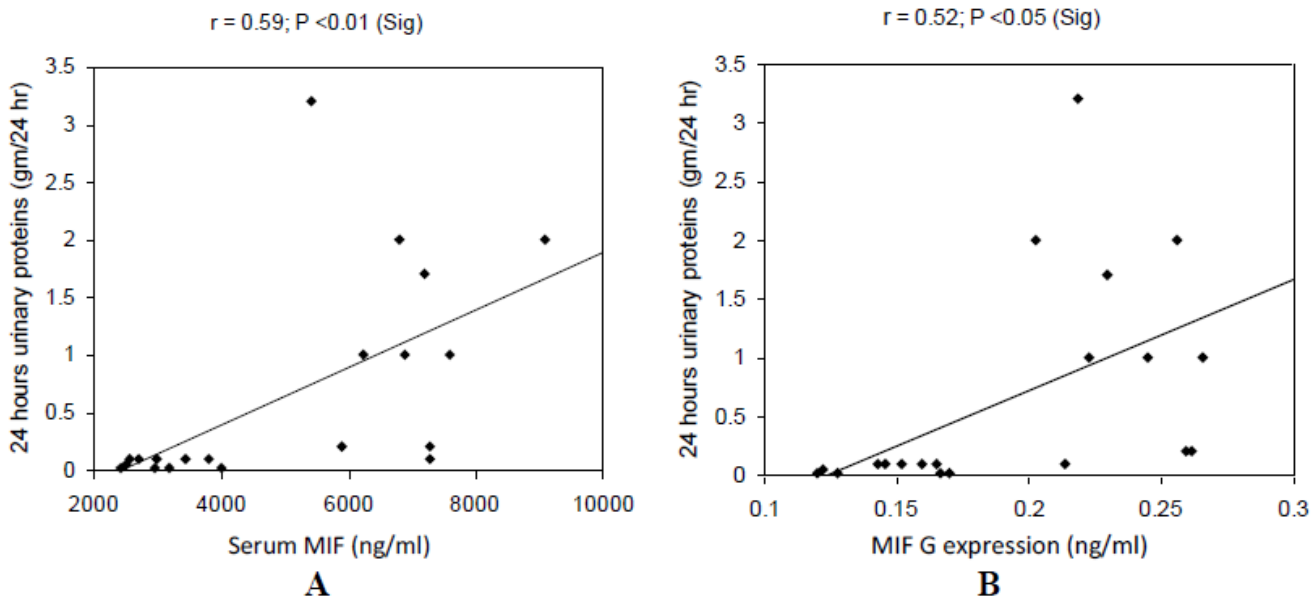

Figure 7: Correlation between the (serum MIF and MIF G expression) and 24 hours urinary proteins among all lupus patients.

Discussion

The aim of this work was to investigate the changes in macrophage migration inhibitory factor mRNA expression in peripheral blood mononuclear cells in relation to disease activity and renal affection in patients with systemic lupus erythematosus.

For this purpose twenty lupus patients, regularly attending the Pediatric Allergy and Immunology Clinic, Children Hospital, Ain Shams University and fulfilling the American Rheumatism Association Revised Criteria for diagnosis of SLE, were studied in comparison to thirty-five healthy subjects as normal controls.

In our study, both serum MIF and MIF gene expression by reverse PCR on macrophages were higher in lupus patients with a mean of serum MIF 22.67 ± 1.46 ng/ml and a mean of MIF gene expression of 0.24 ± 0.02 ng/ml in comparison to the means in healthy controls of 1.71 ± 2.7 ng/ml and 0.0021 ± 0.00015 ng/ml respectively. In a similar way, MIF in serum and by gene expression were higher in lupus patients with clinically evident lupus nephritis in comparison to those lupus patients without clinically evident nephritis with means of 22.67 ± 1.6 ng/ml and 0.23 ± 0.22 ng/ml in comparison to 12.25 ± 0.7 ng/ml and 0.14 ± 0.018 ng/ml respectively. The levels of both serum MIF and MIF gene expression were higher in both patients with and without clinically evident nephritis in comparison to healthy controls.

Our results can be explained by the fact that immunoregulatory abnormalities observed in SLE include hyperresponsive B cells and abnormal antibody production as well as abnormal T cell responses. MIF has been identified as a mediator of activation of B and T cells as well as of synovial cells, endothelium and glomerular cells. In addition, MIF is expressed in inflammatory lesions in organs targeted by SLE including joints, kidney, bowel, skin and brain. The fact that MIF was higher in lupus patients suffering from clinical lupus nephritis than those who did not can be due to the fact that local production of this inflammatory molecule plays an important role in mediating progressive renal injury in human glomerulonephritis.

In agreement with our study, [17] who reported that MIF mRNA expression in peripheral blood mono nuclear cells (PBMC) and serum MIF concentration was significantly higher in patients with SLE and that serum MIF may be a useful parameter for monitoring disease activity in patients with SLE.

Also agreement with Foote et al. [6] results, they found that MIF was over expressed in patients with SLE and suggested an evidence of an association between MIF and lupus related disease damage.

On the other hand, Hoi et al. [23] indicated that MIF by virtue of its pluripotent functions, may be a critical mediator of inflammation and damage in SLE. It may be instrumental in the maintenance of immune dysregulation from perpetuation of immune activation, promoting leucocyte recruitment and cellular inflammation, to maintenance of macrophage effector function in target tissues. Hui et al. [24] found that renal MIF expression is markedly up regulated in severe proliferative forms of human glomerulonephritis and that renal MIF expression gave a highly significant correlation with macrophage and T cell infiltration, histologic damage and renal function suggesting that MIF may be an important mediator of renal injury. Brown et al. [25] indicated that MIF expression is up regulated in lupus nephritis and correlates with the degree of renal dysfunction, histologic damage and leucocyte infiltration.

Interestingly, Leung et al. [26], Matsumoto and Kanmatsuse [27] found that MIF gene expression in cultured human mesangial cells is increased in patients with IgA nephropathy which may play a major pathogenic role in disease activity.

In this study, we showed that serum MIF and MIF gene expression were significantly higher in our group of patients who suffered from either hematuria, edema, hypertension or positive anti-DNA values than other patient who did not experience the previous symptoms.

This finding can be simply explained by the hypothesis that these symptoms are usually associated with evidence of lupus nephritis in which it is more likely for MIF to be elevated since its expression is up regulated in renal affection by lupus. Also, In our study, both serum MIF and MIF gene expression had a positive significant correlation to the values of cumulative steroids dose a finding which can be explained by the fact that the synthesis and release of MIF is increased by corticosteroids *in vitro* and *in vivo*. Moreover, MIF has the ability to override and antagonize the anti-inflammatory effects of corticosteroids.

In agreement with our study, Foote et al. [6] found a significant positive correlation between MIF and steroids dose, particularly at higher doses and that this association remained significant even when the possible confounding effects of variables such as disease damage and serum creatinine were controlled. Also, Morand and Leech [28] indicated that MIF acts as a physiological counter-regulator of endogenous corticosteroids and that in a patient receiving therapeutic corticosteroids, the persistence and induction of MIF would serve to antagonize the therapeutic effects of corticosteroids on inflammation.

Moreover, Fingerle-Rowson et al. [29] strongly supported the hypothesis of MIF as being a glucocorticoid antagonist during stress induced lymphocyte redistribution and suggested the potential utility of inhibiting MIF so as to enhance the anti-inflammatory properties of glucocorticoid while sparing their dose limiting side effects.

In our study, a positive significant correlation was found between serum creatinine and both serum MIF and MIF gene expression, a finding which can be explained by the fact that factors influencing renal function may contribute to serum MIF indicating the overexpression of MIF in active glomerulonephritis. This finding is also supported by Brown et al. [25].

In agreement with our study, Lan et al. [24] found that glomerular and tubular MIF expression gave a highly significant correlation with the loss of renal function and suggested that MIF causes renal injury in human glomerulonephritis by inducing macrophage and T cell accumulation and the subsequent activation of these cells leading to immune cell mediated tissue damage.

In contrary to our results, Foote et al. [6] found an inverse relationship between serum creatinine and MIF and suggested that the kidney may be a significant source of MIF in humans.

Statistical analysis in our results indicated a positive significant correlation between serum MIF and MIF gene expression with both SLEDAI score and ESR in all lupus patients suggesting that MIF is associated with disease severity in SLE.

On the other hand, Foote et al. [6] failed to find a relationship between concurrent serum MIF and disease activity score and explained his finding by the fact that his study group included a mostly abundant community based population with very low disease activity scores which limited the potential of finding this association.

In our study, there was a significant positive correlation between serum MIF and MIF gene expression with 24 hours urinary protein excretion in all lupus patients. This finding can be simply explained by the fact that proteinuria is a picture of lupus nephritis in which MIF expression is expected to increase secondary to organ damage and tissue cellular infiltration.

In agreement with our study, Matsumoto and Kanmatsuse [27] found positive correlation between urinary protein excretion and urinary MIF. They suggested that such correlation could be due to the lower molecular weight of MIF as compared to albumin and hence its ability to pass through pores in glomerular basement membrane.

Finally, our results showed a positive significant correlation between serum MIF and MIF gene expression with the duration of illness in all lupus patients. Such finding could be due to the role of MIF in lupus disease and its correlation to multiple organ damage in lupus which is expected to be aggravated by time and disease duration.

Conclusion

MIF both in the serum and MIF gene expression by reverse PCR in macrophages were significantly higher in all lupus patients in comparison to healthy controls being significantly higher in those lupus patients with clinically evident nephritis than those without. Also MIF had a significant positive correlation to SLEDAI score and ESR indicating its correlation with disease activity.

References

1. Klein-Gitelman MS, Miller ML (2004) Systemic lupus erythematosus. (17th edn.). Nelson textbook of Pediatrics, WB Saunders Company, Philadelphia. pp: 809-813.
2. Bacher M, Metz CN, Calandra T, Mayer K, Chesney J, et al. (1996) An essential regulatory role for macrophage migration inhibitory factor in T-cell activation. Proc Natl Acad Sci USA 93: 7849-7854.
3. Calandra T, Bernhagen J, Mitchell RA, Bucala R (1994) The macrophage is an important and previously unrecognized source of macrophage migration inhibitory factor. J Exp Med 179: 1895-1902.
4. Flaster H, Bernhagen J, Calandra T, Bucala R (2007) The macrophage migration inhibitory factor-glucocorticoid dyad: regulation of inflammation and immunity. Mol Endocrinol 21: 1267-1280.
5. Roger T, Chanson AL, Knaup-Reymond L, Calandra T (2005) Macrophage migration inhibitory factor promotes innate immune responses by suppressing glucocorticoid-induced expression of mitogen-activated protein kinase phosphatase-1. Eul J Immunol 35: 3405-3413.
6. Foote A, Briganti EM, Kipen Y, Santos L, Leech M, et al. (2004) Macrophage migration inhibitory factor in systemic lupus erythematosus. J Rheumatol 31: 268-273.
7. Santos L, Hall P, Metz C, Bucala R, Morand EF (2001) Role of macrophage migration inhibitory factor (MIF) in murine antigen-induced arthritis: Interaction with glucocorticoids. Clin Exp Immunol 123: 309-314.
8. Leech M, Metz C, Bucala R, Morand EF (2000) Regulation of macrophage migration inhibitory factor by endogenous glucocorticoids in rat adjuvant-induced arthritis. Arthritis Rheumatol 43: 827-833.
9. Berdeli A, Mir S, Ozkayin N, Serdaroglu E, Tabel Y, et al. (2005) Association of macrophage migration inhibitory factor -173C allele polymorphism with steroid resistance in children with nephrotic syndrome. Pediatr Nephrol 20: 1566-1571.
10. De Benedetti F, Meazza C, Vivarelli M, Rossi F, Pistorio A, et al. (2003) British Paediatric Rheumatology Study Group: Functional and prognostic relevance of the-173 polymorphism of the macrophage migration inhibitory factor gene in systemic-onset juvenile idiopathic arthritis. Arthritis Rheum 48: 1398-1407.
11. Aeberli D, Yang Y, Mansell A, Santos L, Leech M, et al. (2006) Endogenous macrophage migration inhibitory factor modulates glucocorticoid sensitivity in macrophages via effects on MAP kinase phosphatase-1 and p38 MAPkinase. FEBS Lett 580: 974-981.
12. Baeuerle PA, Henkel T (1994) Function and activation of NF-kappa B in the immune system. Ann Rev Immunol 12: 141-179.
13. Baeuerle PA, Baltimore D (1996) NF-kappa B: Ten years after. Cell 87: 13-20.

14. Verma IM, Stevenson JK, Schwarz EM, Van Antwerp D, Miyamoto S (1995) Rel/NF-kappa B/I kappa B family: Intimate tales of association and dissociation. Genes Dev 9: 2723-2735.
15. Auphan N, Didonato JA, Rosette C, Helmberg A, Karin M (1995) Immunosuppression by glucocorticoids: inhibition of NF-kappa B activity through induction of IkB synthesis. Sci 270: 286-290.
16. Scheinman RI, Cogswell PC, Lofquist AK, Baldwin AS (1995) Role of transcriptional activation of I kappa B alpha in mediation of immunosuppression by glucocorticoids. Sci 270: 283-286.
17. Chen WY, Li GR, Fang F, Yang X, He XS, et al. (2004) The expression of migration inhibitory factor in peripheral blood mononuclear cells and correlation with disease activity in systemic lupus erythematosus. Zhonghua Nei Ke Za Zhi 43: 572-575.
18. Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, et al. (1982) The revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheumatol 25: 1271-1277.
19. Davis ID, Avner ED (2004) Glomerulonephritis associated with systemic lupus erythematosus. (17th edn.). Nelson Textbook of Pediatrics, WB Saunder, Philadelphia. pp: 1743-1744.
20. Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang CH (1992) Derivation of the SLEDAI. A disease activity index for lupus patients. The committee on prognosis studies in SLE. Arthritis Rheumatol 35: 630-640.
21. Matsui Y, Okamoto H, Jia N, Akino M, Uede T, et al. (2004) Blockade of macrophage migration inhibitory factor ameliorates experimental autoimmune myocarditis. J Mol Cell Cardiol 37: 557-566.
22. Sambrook R, Ting Y, Manos MM (1989) PCR protocols: A guide to methods and applications. APMIs 97: 957-970.
23. Hoi AY, Morand EF, Leech M (2003) Is macrophage migration inhibitory factor a therapeutic target in systemic lupus erythmatosus? Immunol Cell Biol 81: 367-373.
24. Lan HY, Yang N, Nikolic-Paterson DJ, Yu XQ, Mu W, et al. (2000) Expression of macrophage migration inhibitory factor in human glomerulonephritis. Kidney Int 57: 499-509.
25. Brown FG, Nikolic-Paterson DJ, Hill PA, Isbel NM, Dowling J, et al. (2002) Urine macrophage migration inhibitory factor reflects the severity of renal injury in human glomerulonephritis. J Am Soc Nephrol 13: S7-S13.
26. Leung JCK, Chan LYY, Tsang AWL, Liu EWL, Lam MF, et al. (2004) Anti-macrophage migration inhibitory factor reduces transforming growth factor-β1 expression in experimental IgA nephropathy. Nephrol Dial Transplant 19: 1976-1985.
27. Matsumoto K, Kanmatsuse K (2002) Urinary levels of macrophage migration inhibitory factor in patients with IgA nephropathy. Nephron 92: 309-315.
28. Morand EF, Leech M (1999) Glucocorticoid regulation of inflammation the plot thickness. Inflamm Res 48: 557-560.
29. Fingerle-Rowson G, Koch P, Bikoff R, Lin X, Metz CN, et al. (2003) Regulation of macrophage migration inhibitory factor expression by glucocorticoids *in vivo*. Am J Pathol 162: 47-56.