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ORIGINAL ARTICLE

Urine macrophage migration inhibitory factor in pediatric systemic lupus erythematosus

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Abstract We reported a series of ten patients with lupus nephritis (five patients in the relapse phase and five in the remission phase) and measured the macrophage migration inhibitory factor (MIF), an important pro-inflammatory cytokine with probable role in the pathogenesis of many inflammatory diseases, in their urine samples. MIF/creatinine (Cr) ratio directly correlated with disease activity and it does not have any significant difference between inactive disease and normal ones. We found that the urine MIF/Cr ratio not only differentiates active disease from inactive disease and normal ones but also correlates with the activity indices of renal pathology.

Keywords Activity index · Lupus nephritis
Macrophage migration inhibitory factor

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease that may involve nearly every organ system in an inflammatory process. Although the origin of SLE is not yet known, increasing evidence indicates that it is caused, or at least influenced, by a combination of genetic, immunologic, homoral, and possibly environmental factors. Immune-mediated nephritis is a common complication of SLE. It is now clear that multiple independent mechanisms contribute to disease onset and pathogenesis including nephritogenic autoantibodies, activated macrophages, and T cells and abnormal production of macrophage growth factors and cytokines [1].

Macrophage migration inhibitory factor (MIF) is an important pro-inflammatory cytokine found to be expressed at sites of inflammation. The profile of activities of MIF in vivo and in vitro is strongly suggestive of a role for MIF in the pathogenesis of many inflammatory diseases, including SLE, and hence, antagonism of MIF is suggested as a potential therapeutic strategy in inflammatory disease [2–5].

Our aim was to find associations between urine MIF and disease activity in patients with SLE.

Materials and methods

Patients In a prospective study, all the patients admitted with the diagnosis of SLE in Aliasghar Children's Hospital, Tehran, Iran between July 2005 and February 2007 were included (n=10, mean age=11.3 years). No patient had evidence of urinary tract infections during this period. Five patients were in the relapse phase confirmed by high serum levels of anti-nuclear antibody (ANA), anti-double strand DNA antibody (anti-dsDNAab), and erythrocyte sedimentation rate (ESR) and low levels of complement, while five patients were in the remission phase (Table 1). For the controls, 12 normal healthy volunteers, age- and sex-matched with cases donated urine specimens for analysis.

Urine samples Sterile midstream urine samples were collected and stored at 4°C for a maximum of 6 h before processing. The urine was centrifuged at 1,500×g for 10 min

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Table 1 Clinical features of patients with SLE

Variables	Case number									
	1	2	3	4	5	6	7	8	9	10
Age (years)	11	14	9	12	7	15	10	9	12	14
Sex	F	F	F	F	F	F	M	F	F	F
Classification[a]	IV	IV	V	IV	V	IV	IV	V	IV	IV
Urine MIF (pg/ml)	5,031	4,649	1,960	4,722	4,612	18	55	145	75	64
Urine Cr (μmol/l)	196	46	93	102	195	186	138	295	65	42
MIF/Cr (pg/μmol Cr)	25.67	101.1	21.1	46.3	23.65	0.1	0.4	0.49	1.15	1.52
Serum Cr (mg/dl)	3.4	0.7	0.6	0.5	0.6	1	0.6	0.7	0.8	0.5
ANA (1:)	640	160	1,280	320	1,280	320	160	320	320	160
Anti-dsDNAab	+	+	+	+	+	–	–	–	–	–
C_3	↓	↓	↓	↓	↓	NL	NL	NL	NL	NL
C_4	↓	↓	↓	↓	↓	NL	NL	NL	NL	NL
CH50 (U/ml)	↓	↓	↓	↓	↓	NL	NL	NL	NL	NL
ESR	108	105	92	100	95	10	20	15	17	14
Phase	rel	rel	rel	rel	rel	rem	rem	rem	rem	rem

MIF: macrophage migration inhibitory factor, *Cr*: creatinine, *WBC*: white blood cell, *PLT*: platelet, *ANA*: anti-nuclear antibody, *Anti-dsDNAab*: anti-double strand DNA antibody, *ESR*: erythrocyte sedimentation rate, *rel*: relapse, *rem*: remission.

[a] World Health Organization classification system for lupus nephritis.

to separate debris and then a protease inhibitor cocktail (Sigma, Castle Hill, NSW, Australia) was added (5 μl/ml) before storage at −80°C. A 1-ml aliquot was analyzed for urine creatinine.

MIF ELISA Urine MIF concentration was quantitated by enzyme-linked immunosorbent assay (ELISA) methods according to the manufacturer's instructions (R & D Systems, Minneapolis, MN, USA). Costar 96-well EIA plates (Cat. No. 2592) were coated overnight with 100 μl per well of 2 μl/ml mouse anti-human MIF capture antibody. Wells were washed 3 times with 400 μl per well of 0.05% Tween 20 in phosphate-buffered saline (PBS) (wash buffer) and then blocked by adding 300 μl per well of 1% bovine serum albumen (BSA) in PBS for a minimum of 1 h. Urine samples and recombinant MIF standards (prepared in serial dilutions using 1% BSA in PBS) were added 100 μl per well. The plates were incubated at room temperature for 2 h. Then, the plates were washed 3 times with wash buffer, and bound MIF was detected by a 2-h incubation with 100 μl per well of 200 ng/ml biotinilated goat anti-human MIF antibody diluted in 1% BSA in PBS. After 3 times washing, the plates were incubated with 100 μl per well of working dilution of streptavidin-HRP for 20 min at room temperature, washed again 3 times with wash buffer, and then incubated for 20 min with 100 μl per well of ready to use 3,3′,5,5-tetramethylbenzidine (TMB). The colorimetric reaction stopped by the addition of 50 μl of 0.5 M H_2SO_4. The optical density of each well at 450/630 nm was measured using a microplate reader.

Results

We included ten patients with the diagnosis of SLE in this study (Table 1). Five patients (mean age=10.6 years, all females) were in the relapse phase according to the positive anti-nuclear antibody (ANA) and anti-double strand DNA antibody (anti-dsDNAab), high erythrocyte sedimentation rate (ESR), and low CH50, C_3, and C_4. Mean urine MIF/Cr ratio was 43.56 pg/μmol creatinine in this group. The other 5 patients (mean age=12 years, female/male ratio=4/1) were in the remission phase according to the low ESR, CH50, C_3, and C_4. Mean urine MIF/Cr ratio was 0.73 pg/μmol creatinine in this group. For the controls, 12 normal healthy volunteers (mean age=11.1 years, all females) were included. Mean urine MIF/Cr ratio was 1.9 pg/μmol creatinine in this group.

Discussion

MIF is a broad spectrum pro-inflammatory cytokine. It is an important inflammatory mediator in the perpetuation of immune activation in SLE. In animal models of SLE, T cell activation is an absolute requirement for the full expression of the disease. The importance of MIF in T cell activation and the development of animal models of SLE suggests the possibility that MIF could be relevant to the pathogenesis of this disease [6]. MIF also could upregulate adhesion molecules and promote leukocyte recruitment in the development of SLE [6].

Apoptosis is a tightly regulated process of programed cell death that has been impaired in SLE. Existing data strongly support the contention that MIF exerts anti-apoptotic effects in vitro and in vivo, which would be consistent with the possible impairment of apoptosis in SLE. Brown et al. found a significant 3.4-fold increase in urine MIF concentration in proliferative nephropathies such as IgA glomerulonephritis, crescentic GN, SLE World Health Organization class IV [7]. Mizue et al. found a fourfold increase in serum MIF levels in patients with SLE [8]. In addition, evidence suggests that MIF may be a clinical marker in SLE and serum levels of MIF correlate with indices of organ damage in lupus patients. MIF expression correlates with the degree of renal dysfunction, histological damage, and leukocyte infiltration. It is believed that increased serum MIF reflects systemic symptoms and increased urine MIF is seen in primarily renal involvement without systemic disease [7].

In our study the mean urine MIF/Cr ratio in patients with active disease was 43.56 pg/μmol creatinine in contrast to 0.73 pg/μmol creatinine in patients with inactive disease and 1.9 pg/μmol creatinine in the controls. Thus, MIF/Cr ratio directly correlated with SLE disease activity and it does not have any significant difference between inactive disease and normal ones (Table 1).

Microscopic findings of renal biopsies in cases 2 and 4 (Table 1) were wire loop, endocapillary proliferation and cellular crescent pattern, which favor high activity indices. The mean urine MIF/Cr ratio in these patients was 73.7 pg/μmol creatinine in contrast to 23.47 pg/μmol creatinine in the remaining 3 patients in the relapse phase. So, we found that urine MIF/Cr ratio not only differentiates active disease from inactive disease and normal ones but also correlates with the activity indices of renal pathology.

We concluded that urine MIF/Cr ratio directly correlates with SLE activity and also with its renal pathology activity index. In our opinion, following this case series, it is necessary to measure MIF/Cr ratio in a study population large enough to see a statistically significant difference.

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