Macrophage Migration Inhibitory Factor in Systemic Lupus Erythematosus and its Expression in Lupus Nephritis

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Abstract

Objective: the aim of this study was to access the potential involvement of MIF in SLE, its relationship with corticosteroid dose, also, to measure serum and urinary MIF levels in SLE as well as detecting renal MIF expression in SLE GN.

Methods: Serum and urine MIF concentrations were measured by enzyme-linked immunosorbent assay in 20 SLE female patients with lupus nephritis, World Health Organization class II, III, IV, with mean age of 35.15±10.42 years and in 10 normal healthy, age matched, female volunteers. All patients were subjected to detailed clinical assessment and laboratory investigations. Serum and urinary MIF concentrations were measured by ELISA technique. Renal MIF expression was assessed by immunostaining of biopsy tissue. Univariate and multivariate regression analysis were used to examine the associations between serum and urine MIF concentrations, renal MIF expression, disease-related indices of SLE and corticosteroid use.

Results: A statistically significant 2.98-fold-increase was detected in mean urinary MIF (U MIF) levels in SLE patients compared to controls. While, mean Serum MIF (S MIF) showed no significant difference between cases & control. Both S & U MIF concentrations were positively correlated with SLICC/ACR DI but not with SLEIDAI. Corticosteroid doses showed a highly positive correlation with S MIF, serum creatinine & SLICC/ACR DI. Also a positive correlation was found between the different histopathologic grades of renal affection & the U MIF. Immunohistochemistry staining of all normal kidney specimens showed that MIF is constitutively weakly expressed by some glomerular & parietal epithelial cells & by most tubular epithelial cells. In contrast, there was a significant increase in glomerular & tubular MIF protein staining in SLE nephropathy. This increased MIF expression correlated positively with both S MIF and U MIF, SLICC/ACR DI & the daily steroid dose.

Conclusion: This study shows that serum MIF is over-expressed in SLE patients and that the urine MIF concentration is significantly increased in SLE World Health Organization class IV patients and correlates with the degree of renal injury. Thus, urine MIF levels reflect MIF expression within the kidney.

Key words: SLE, MIF, GN, renal biopsy.

Introduction

Originally described in 1966, macrophage migration inhibitory factor (MIF) was initially identified as a 12.5-kD protein secreted by activated T lymphocytes capable of inhibiting random migration of macrophages, concentrating macrophages at inflammatory loci, and enhancing their ability to kill intracellular parasites and tumour cells (David,1966). Recent data indicate that other types of cells, such as macrophages, endothelial cells, and fibroblasts, can produce MIF (Steinhoff et al., 1999), and many other functions have been attributed to this molecule, such as the
regulation of cell growth, including tumourigenesis, T cell activation, and angiogenesis (Lolis, 2001). MIF is constitutively expressed by B cells, and antagonism of MIF inhibits B cell proliferation (Chesney et al., 1999). It is also produced by T cells stimulated by recall antigens, mitogens, and anti-CD3 antibodies, and antagonism of MIF prevents T cell activation by these factors (Bacher et al., 1996). Furthermore, recent reports suggest that MIF has a critical role in inflammatory and immune responses (Metz and Bucala, 1997). In particular, MIF has been shown to induce the synthesis of pro-inflammatory cytokines, including tumour necrosis factor-α (TNF-α), interleukin (IL)1 and IL8 in immunocompetent cells. Moreover, it has also been verified that MIF acts as a powerful stimulator for nitric oxide production (Liew, 1994).

MIF is the only molecule described that can override the anti-inflammatory action of glucocorticoids (Calandra et al., 2000). Moreover, in animal models and in vitro, MIF has the unique ability to exert an antagonistic effect on corticosteroid suppression of immune inflammation (Santos et al., 2001). This is in contradiction with the role of MIF as a pro-inflammatory cytokine, but it supports the concept that physiologic levels of glucocorticoids regulate the immune inflammatory response (Amoli et al., 2002). MIF also acts in a dose dependent manner in regulating the inhibitory effects of glucocorticoids in the immune system (Donnelly and Bucala, 1997). MIF is inhibited by pharmacological concentrations of glucocorticoids. However, at low concentrations these drugs increase the synthesis of MIF (Leech et al., 2000). The hypothesis that MIF operates as a physiological counter-regulator of corticosteroids (Fingerle-Rowson et al., 2003) suggest that therapeutic antagonism of MIF may have specific steroid-sparing benefits, by increasing the immunosuppressive and anti-inflammatory properties of endogenously released glucocorticoids, thus reducing the requirement for steroid therapy in a variety of autoimmune and inflammatory conditions (Bucala, 1998).

As mentioned in several previous reports, an essential role for MIF has been established in the tuberculin delayed-type hypersensitivity reaction (Bernhagen et al., 1996), in several inflammatory skin diseases (Steinhoff et al., 1999), in wound healing process (Abe et al., 2000), in immune-mediated diseases of the central nervous system (Niino et al., 2000) and in inflammatory bowel disease (De Jong et al., 2001).

MIF potentiates lethal endotoxemia in mice and can overcome glucocorticoid-mediated suppression of lethal endotoxemia (Calandra et al., 2000). A pathologic role for MIF has also been established in experimental models of arthritis (Mikulowska et al., 1997), where MIF showed a pivotal role in the pathogenesis of rheumatoid arthritis (RA) (Leech et al., 1998 and 2003) and glomerulonephritis (GN) (Lan et al., 1997a) which has led to increasing acceptance of MIF as a key cytokine in chronic inflammatory diseases. The administration of specific anti-MIF monoclonal antibodies decrease arthritis disease expression (Leech et al., 2000) and inhibit the severity as well as frequency of disease (Sampey et al., 2001).

The dysregulation of MIF has recently been described in several inflammatory diseases (Meazza et al., 2002). Leech et al. (1999) demonstrated the high expression of MIF in inflamed synovial tissue from RA patients, with a unique up and down regulation, respect-ively, induced by low and high glucocorticoid concentrations. Morand et al. (2002) found a strong correlation between the synovial MIF and disease activity which corroborates existing evidence of the role of this cytokine in RA. These findings were recently confirmed by Onodera et al. (2004) who explained the migration of inflammatory cells into the synovium of rheumatoid joints to be due to induction of IL8 and IL1-beta mRNA which are up regulated by MIF. Meazza et al. (2002) reported that MIF is a relevant cytokine in the pathogenesis of juvenile idiopathic arthritis (JIA), particularly in systemic-onset JIA. Also, in a recent study done by Donn et al. (2004) a functional promoter
haplotype of MIF was found to be linked and associated with JIA. Sampy et al. (2001) showed that MIF exerts an up regulation of fibroblast-like synoviocyte phospholipase A2 and cyclooxygenase 2. Selvi et al. (2003) found a significantly higher serum concentration of MIF in patients with diffuse systemic sclerosis. Also, they found a significantly higher MIF level in the fibroblast cultures. Of particular interest in SLE is the observation that MIF is the only proinflammatory cytokine that is induced rather than suppressed by corticosteroids (Leech et al., 1999). This was confirmed by Foote et al. (2004) who found that serum MIF was overexpressed in SLE patients.

SLE is a chronic multisystem autoimmune disease with an unknown etiology, characterized by abnormalities of immune-inflammatory system function including altered B and T cell function, and by inflammation of organs including joints and kidneys (Lipsky, 2001). Corticosteroids are a mainstay of the treatment of SLE, despite their widely known side effects.

In the kidney, MIF is weakly expressed by some glomerular epithelial cells and by approximately half of the cortical tubules (Lan et al., 1998). Renal MIF mRNA and protein expression is upregulated in different types of experimental kidney disease, including crescentic anti-glomerular basement membrane GN (Tesch et al., 1998). In each of these disease models, up-regulation of MIF expression is closely associated with macrophage accumulation and tissue damage. Tang et al. (1994) reported that induction of the early renal injury in this disease model is largely attributed to the early and transient neutrophil influx after the deposition of antibody and complement on the glomerular basement membrane. In contrast, the subsequent progression of renal injury is mediated by macrophages and T cells, which supports the concept that MIF is a key regulator of immune disease mediated by macrophages and T cells (Lan et al., 1997b). Administration of a neutralizing anti-MIF antibody inhibited macrophage and T cell accumulation and histologic damage, reduced proteinuria, and prevented renal dysfunction in rat crescentic anti-glomerular basement membrane disease (Lan et al., 1997b). Furthermore, administration of the anti-MIF antibody was shown to partially reverse the progressive phase of established crescentic disease in rats (Yang et al., 1998).

Analysis of renal MIF expression in human biopsy tissue revealed that renal MIF expression is upregulated in proliferative forms of GN. Renal MIF expression significantly correlates with renal dysfunction, histologic damage, and leukocytic infiltration (Lan et al., 2000). Taken together with data from functional blocking studies in the rat. These data suggest that MIF plays an important role in the pathogenesis of human proliferative GN and could be an attractive target in the treatment of progressive human GN.

Despite the accumulation of evidence for a key role for MIF in autoimmune-inflammatory diseases, MIF has not been extensively investigated in SLE. Based on the hypothesis that urinary MIF excretion may reflect the level of MIF production within the kidney, which may furthermore reflect the degree of renal injury, the aim of this study was to access the potential involvement of MIF in SLE, its relationship with corticosteroid dose, also, to measure serum and urinary MIF levels in SLE as well as detecting renal MIF expression in SLE GN.

**Material and Methods**

**Patients**

Twenty female SLE patients who fulfilled the American criteria for classification of SLE (Tan et al., 1982) with lupus nephritis WHO class II, III, IV were recruited from the outpatient clinic of Rheumatology & Rehabilitation and nephrology departments at Ain Shams University Hospital and were enrolled in this study. Their mean age was 35.15±10.42 years (range18-53 years).

Ten age-matched apparently healthy female volunteers, with no history of acute or chronic inflammatory disease, were enrolled from the department personnel as
controls. Their mean age was 33.2±11.43 years (range 19-50 years).

**All patients and controls were subjected to:**  
**Clinical evaluation**  
All patients had a detailed clinical assessment for involvement of the internal organs and were evaluated for the presence of gastrointestinal, pulmonary, cardiac, renal, or musculoskeletal affection.

Disease damage was measured using Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index (SLICC/ACR DI) (Gladman et al., 1996) and disease activity was assessed using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) (Bombardier et al., 1992). Patients were classified as inactive if their SLEDAI score was less than 5, and active if their SLEDAI score was more than 10. Each patient was assessed for concomitant medication and corticosteroid dose was recorded as the daily dose of Prednisolone (mg/day) at the time of the study.

**Laboratory assessment**

**Routine lab works include**
1. Complete blood picture (CBC) by Coulter counter.
2. Erythrocyte sedimentation rate (ESR) by Westergren method.
3. Antinuclear antibodies (ANA).
4. Anti-dsDNA using indirect immunofluorescent antibody test.
5. Kidney function tests: including serum creatinine and proteinuria.
6. Complete urine analysis for casts, hematuria, and pyuria.

**Serum Samples**
All collected blood samples were limited between 9 am and 12 pm. Blood was withdrawn by venipuncture in plain tubes and left at room temperature for 1 hr rto clot before being stored at 4°C for up to 4 hr. The blood then was centrifuged at 1500 x g for 10 min. The serum was formed into aliquots and stored at -80°C. Only one freeze-thaw cycle was allowed.

**Urine Samples**
Sterile midstream urine samples were collected from patients and then were stored at 4°C for a maximum of 12 hr before processing. A 1-ml aliquot was analyzed for urine creatinine and proteinuria. The urine was centrifuged at 1500 x g for 10 min to separate debris and a protease inhibitor cocktail (Sigma, Castle Hill, New South Wales, Australia) was added; then urine was formed into aliquots and stored at -80°C.

**Serum and urine MIF Enzyme-Linked Immunosorbent Assay**
Serum and urine MIF concentrations were quantitated by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer’s instructions (R&D Systems, Minneapolis, MN). In brief, ELISA plates were coated overnight with 2 µg/ml (100µl/well) mouse anti-human MIF capture antibody and incubated overnight at room temperature. Wells were washed 3 times with washing solution (10 mM PBS; pH 7.4, 0.05% (wt/vol) Tween-20), blocked by 300µl of blocking solution (10 mM PBS; pH7.4, 1% (wt/vol) bovine serum albumin (BSA), 5% (wt/vol) sucrose, and 0.05% NaN₃ in PBS) for 2 hr. Test samples (human serum, human urine, or recombinant MIF standards) diluted in Tris buffered saline-BSA (0.1% BSA, 0.05% Tween-20, 20 mM Tris-HCl, 150 mM NaCl, pH 7.3), were added in triplicate (100µl/well) and then were incubated at room temperature for 2 hr. After washing with PBST, bound MIF was detected by a 2-hr incubation with 200 ng/ml biotinylated anti-human MIF antibody diluted in 0.1% BSA, 0.05% Tween-20 in 20 mM Tris-HCl, and 150 mM NaCl, pH 7.3. After washing, samples were incubated with 1.25 ng/ml peroxidase-conjugated streptavidin (Zymed, South San Francisco, CA) for 30 min, washed in PBST, and then incubated for 30 min with 100 µl/well ready-to-use 3,3',5,5'-tetramethylbenzidine (Zymed) and the colorimetric reaction stopped after 20 minutes by the addition of 0.5 M H₂SO₄. The adsorption was measured at 450/570 nm with a microplate reader. MIF concentration was expressed as pg/ml for
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serum or pg MIF/µmol for urine. The sensitivity limit was 18 pg/ml.

MIF is stable in human urine; the ELISA measurements are constant for up to 24 hr when stored at 4°C or room temperature. The MIF ELISA assay is highly reproducible, and when we analyzed samples (in triplicate) up to 12 times, the SD was 6.6% of the mean value.

Renal biopsies and histopathology

Renal biopsies were taken from 7 female SLE patients, World Health Organization class II, III and IV. In addition, five normal human kidney specimens were analyzed (from unaffected areas of nephrectomy for renal cell carcinoma).

Sections (4 µm) of formalin-fixed, paraffin-embedded biopsy tissue were stained with hematoxylin and eosin. The percentage of glomeruli exhibiting crescent formation was scored in 10-30 glomerular cross sections (gcs) per biopsy. Glomerular hypercellularity was assessed as follows: 0 = normal (less than 60 cells/gcs); 1 = mild (60-90 cells/gcs); 2 = moderate (90-120 cells/gcs); and 3 = severe (more than 120 cells/gcs).

Antibodies

Mouse monoclonal antibodies (MoAb) used for immunostaining were as follows: IIID9, mouse MoAb raised against recombinant mouse MIF that cross-reacts with human MIF; UCHL1, mouse anti-CD45RO, which recognizes mature, activated T cells and a subset of resting T cells (Smith et al., 1986); and KP1, mouse anti-CD68, which labels most monocytes and macrophages (Pulford et al., 1989). Peroxidase and alkaline phosphatase–conjugated goat anti-mouse IgG, mouse peroxidase-conjugated anti-peroxidase complexes, and mouse alkaline phosphatase-conjugated anti-alkaline phosphatase complexes were purchased from Dakopatts (Glostrup, Denmark).

MIF expression using Immunohistochemistry Staining:

Two-color immunohistochemistry staining was performed as described previously (Lan et al., 2000). Paraffin sections (4 µm) were treated with 10-min microwave oven heating in 10 mM sodium citrate, pH 6.0, at 2450 MHz and 800 W. Sections then were preincubated with 10% fetal calf serum and 10% normal goat serum in PBS for 20 min, drained, and incubated with KP1 or UCHL1 MoAb overnight at 4°C. Sections then were washed in PBS, endogenous peroxidase inactivated in 0.3% H2O2 in methanol, incubated with peroxidase-conjugated goat anti-mouse IgG, washed in PBS, incubated with mouse peroxidase-conjugated anti-peroxidase complexes, and developed with 3,3-diaminobenzidine to produce a brown colour. Slides then underwent a second microwave treatment to denature the bound Ig and prevent antibody cross reactivity (Lan et al., 1995). Sections then were preincubated with 10% fetal calf serum and 10% normal goat serum in PBS for 20 min, followed by 10% bovine serum albumin in PBS for 20 min, washed, and labeled with the anti-MIF MoAb overnight at 4°C. After washing in PBS, sections then were incubated sequentially with alkaline phosphatase-conjugated goat anti-mouse IgG and mouse alkaline phosphatase-conjugated anti-alkaline phosphatase complexes and then developed with Fast Blue BB Salt (Ajax Chemicals, Melbourne, Australia). Sections were counterstained with periodic acid–Schiff (minus hematoxylin) and mounted in an aqueous medium.

Quantitation of Immunohistochemistry Staining

The number of immunostained cells were counted under high-power microscope fields (x400) in all glomeruli (10-30) for each biopsy and expressed as cell per gcs. The number of KP1-positive and UCHL1-positive interstitial cells was counted in high-power fields of the cortex with a 0.02-mm² graticule fitted in the eyepiece of the microscope for the entire biopsy and expressed as cells per square millimeter. No adjustment of the interstitial cell count was made for tubules or the luminal space. Cortical tubular MIF staining was scored from the entire cortex of the biopsy and
expressed as the percentage of positive tubules. Data are expressed as means ± SD. **Statistical Analysis**

Results are expressed as mean ± SD. Comparisons were made by student t-test for independent groups and by paired samples t-test for dependent groups. Correlations between variables were tested by Pearson correlation coefficient, p<0.05 was considered statistically significant.

**Results**

Twenty SLE female patients, with mean age of 35.15±10.42 years (range18-53 years), mean disease duration of 3.68 ±2.45 years (range 0.5- 10 years) were enrolled in this study. The results of lab works showed that 15 patients had positive ANA (75 %), 17 patients had positive anti-dsDNA (85 %). The mean serum creatinine levels of patients was 1.8 ± 1.69 mg/dl. Patients showed a mean SLEDAI score of 5.55±2.54 and a mean SLICC/ACR DI of 3.15±2.01. The mean steroid dose for patients was 25.13±19.59 mg/day. Four renal biopsies taken from patients showed SLE WHO class IV, two biopsies showed class III, while one showed class II. Clinical and laboratory data are shown in table 1.

Our Biochemical results showed that there was a statistically significant 2.98-fold increase in mean urinary MIF (U MIF) levels in SLE patients (621.35± 330.65 pg MIF/μmol, range 148 to 1290) than in controls (208.5±102.53, range 53 to 420 pg MIF/μmol) P< 0.05. While, mean serum MIF (S MIF) showed no statistical significant difference between cases (949.4±693.48, range 210 to 2005 pg/ml) and control (599.9±460.08, range 105 to 1760 pg/ml) P>0.05

Also, there was a positive correlation between disease damage index SLICC/ACR DI and both S MIF (r=0.82, P<0.05) (fig 1A), and U MIF (r=0.92, P<0.05) (fig 1B). However, disease activity (SLEDAI) scores did not show any correlation with either S MIF (r= -0.18 P=0.44), or with U MIF (r= -0.25 P=0.29).

A statistically significant higher mean level of serum creatinine was found in SLE patients (1.8±1.69mg/dl) compared to control (0.5±0.23mg/dl) P< 0.05. There was also a positive correlation between S MIF and S creatinine P<0.05 (Fig 2)

Corticosteroid doses showed a mean of 25.13 ± 19.59 mg/day in SLE patients and showed a highly positive correlation with both S MIF (r=0.95, P<0.0001) and serum creatinine (r= 0.9, P<0.0001 (Fig 3). There was also a highly positive correlation between steroid dose and SLICC/ACR DI (r=0.77, P<0.0001).

Our histopathologic results of the 7 renal SLE specimens indicated that four renal biopsies showed SLE WHO class IV, two biopsies showed class III, while one showed class II glomerulonephritis (GN). Not only did the mean urinary MIF (U MIF) levels in SLE patients showed a statistically significant 2.98-fold increase (621.35± 330.65 pg MIF/μmol) than in controls (208.5±102.53pg MIF/μmol), but also a positive correlation was found between the different grades of renal affection and the U MIF (r=0.82, P<0.05. figure 4).

Also, three of the 7 biopsies examined showed severe glomerular hypercellularity (>120 cells/gcs), 3 showed moderate hypercellularity (90-120 cells/gcs), while one showed mild hypercellularity (60-90 cells/gcs). The detected glomerular hypercellularity highly positively correlated with both S MIF (r=0.85, P=0.02; Fig 5A) and U MIF (r=0.89, P=0.007; Fig 5B).

Immunohistochemistry staining of all normal kidney specimens showed that MIF is constitutively weakly expressed by some glomerular and parietal epithelial cells and by most tubular epithelial cells. In contrast, there was a significant increase in glomerular and tubular MIF protein staining in SLE nephropathy (Fig 6). This increased MIF expression correlated positively with both S MIF (r=0.89, P=0.007) (Fig 7A), and U MIF (r=0.82, P=0.02) (Fig 7B). MIF expression also correlated positively with SLICC/ACR DI (r=0.89, P=0.008) (Fig 8A), and the daily steroid dose (r=0.82, P=0.02) (Fig 8B), but not with SLEDAI (r= -0.46, P=0.3). Again, studying the correlation between the hypercellularity and either the SLICC/ACR or the steroid dose,
we found a positive correlation between hypercellularity and both variables \( r=0.95, 0.87; P=0.001 \) and 0.01, respectively\) (Fig 9 A and B).

Table (1): Showing the clinical and laboratory data of both groups

<table>
<thead>
<tr>
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<th>SLE cases (N=20)</th>
<th>Controls (N=10)</th>
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<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
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<tr>
<td>Age</td>
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<td>10.42</td>
<td>33.20</td>
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<td>S creatinine</td>
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<td>1.69</td>
<td>0.5</td>
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<tr>
<td>SLICC/ACR DI</td>
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<td>599.9</td>
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<td>208.5</td>
<td>102.53</td>
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<td>GN grade</td>
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<td>MIF expression</td>
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<td>0.79</td>
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Figure (1): Correlation between disease damage index (SLICC/ACR DI) and both A) serum and B) urinary MIF

A) disease damage index (SLICC/ACR DI) with S MIF

\[ r=0.82 \text{ P}=0.00001 \]

B) disease damage index (SLICC/ACR DI) with U MIF

\[ r=0.92 \text{ P}<0.0001 \]
Figure (2): Showing the correlation between S MIF and S creatinine

\[
\begin{align*}
S\text{ MIF} & \quad r=0.86 \quad P<0.0001 \\
S\text{ creatinine} & \\
\end{align*}
\]

Figure (3): Correlation between steroid dose and A) S MIF and B) serum creatinine

A) steroid dose with S MIF

\[
\begin{align*}
S\text{ MIF} & \quad r=0.95 \quad P<0.0001 \\
\text{steroid dose} & \\
\end{align*}
\]

B) steroid dose with serum creatinine

\[
\begin{align*}
S\text{ creatinine} & \quad r=0.90 \quad P<0.0001 \\
\text{steroid dose} & \\
\end{align*}
\]
Figure (4): Correlation between different grades of renal affection and U MIF

![Correlation between different grades of renal affection and U MIF](image)

Figure (5): Correlation between glomerular hypercellularity in renal specimens and A) S MIF and B) U MIF

A) Glomerular hypercellularity with S MIF

![Glomerular hypercellularity with S MIF](image)

B) Glomerular hypercellularity with U MIF

![Glomerular hypercellularity with U MIF](image)
Figure (6): Double immunohistochemistry staining of MIF (blue) and leukocytic infiltration (brown) in SLE nephritis showing marked constitutive MIF expression by some glomerular cells and approximately half of the cortical tubules in association with prominent focal accumulation of macrophages.

Figure (7): Correlation between MIF expression in renal specimens and A) S MIF and B) U MIF

A) MIF expression with S MIF

B) MIF expression with U MIF
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Figure (8): Correlation between MIF expression in renal specimens and A) SLICC/ACR DI and B) steroid dose

A) MIF expression with SLICC/ACR DI
MIF expression r=0.89 P=0.008

B) MIF expression with steroid dose
MIF expression r=0.82 P=0.02

Figure (9): Correlation between glomerular hypercellularity in renal specimens and A) SLICC/ACR DI and B) steroid dose

A) Glomerular hypercellularity with SLICC/ACR DI
Hypercellularity r=0.95 P=0.001

B) Glomerular hypercellularity with steroid dose
Hypercellularity r=0.87 P=0.01

Discussion

Although the etiology of SLE remains unknown, it is clear that patients with SLE have a wide variety of immunoregulatory abnormalities leading to autoimmune mediated organ injury. Immunoregulatory abnormalities observed in SLE include hyperresponsive B cells and abnormal antibody production, as well as abnormal T cell responses (Lipsky, 2001). MIF has been identified as a mediator of activation of B and T cells (Chesney et al., 1999), as well as of synovial cells, endothelium, and glomerular cells (Sampey et al., 2001). In addition, MIF is expressed in inflammatory lesions in organs targeted by SLE including joints, kidney, bowel, skin, and brain. It is increasingly accepted that MIF contributes to the pathogenesis of autoimmune inflam-
matory diseases including RA, immune
glomerulonephritis, inflammatory bowel
disease, psoriasis, and multiple sclerosis
(Leech et al., 2000).

The range of effects of MIF in the
immune system, and its expression in target
organs of SLE, led us to the hypothesis that
MIF is involved in the pathogenesis of
inflammatory organ injury in SLE. Our
results show that patients with SLE were
more likely to have elevated serum MIF
concentrations than controls. This contr-
adicts the report done by Mizue et al
(2000), in which a fourfold increase in
serum MIF levels was described in patients
with SLE. Unfortunately, this report gave
no patient details, so that the severity and
systemic symptoms in these SLE cases are
unknown, thus making it difficult to
compare with the patient group in this
study. One possible explanation for the
apparent discrepancy is that increased
serum MIF reflects systemic symptoms
because the SLE patient group in this study
had primarily renal involvement without
systemic disease. Further studies are needed
to clarify this issue.

Disease related damage, as measured
using the SLICC/ACR DI was greater in
patients with higher serum MIF concen-
tration. This association was independent of
current corticosteroid dose. Similarly,
patients with high SLICC/ACR DI scores
were more likely to have abnormally
elevated values of serum MIF. These data
suggest that serum MIF is associated with
disease severity in SLE. Possible
explanations of this could include MIF gene
polymorphisms, such as those recently
described in patients with inflammatory
arthritis (Baugh et al., 2002).

We were unable to find a relationship
between serum MIF and disease activity
score, as our study included patients with
very low disease activity scores.

Our analysis of the relationship
between serum MIF and corticosteroid use
in SLE patients showed that corticosteroid
use was positively associated with serum
MIF, particularly at higher doses, which
confirms the results reported by Foote et al
(2004).

This study demonstrates that MIF is
readily detected in the urine of normal,
healthy volunteers. Urinary MIF was in-
creased about 2.98 fold over normal levels in
the SLE patients. The urine MIF concen-
tration correlated with the degree of renal
dysfunction, histologic damage, leukocytic
infiltration, and renal MIF expression.

The increase in urinary MIF
concentration seen in SLE patients is
probably the result of increased local
production and secretion of MIF within the
injured kidney. This postulate is supported
by two findings. There was a significant
correlation between renal MIF expression
assessed by immunohistochemistry staining
and the urine MIF concentration. MIF
expression was increased in the glomerulus
(resident and infiltrating mononuclear cells)
and in tubular epithelial cells, both potential
sites of MIF secretion into the urinary
space. In support of this concept, Rice et al.
(1999) have reported that interferon
gamma, a cytokine implicated in the devel-
opment of kidney disease, can induce rapid
secretion of MIF by mesangial cells and
tubular epithelial cells in vitro and
suggested in a recent study done in 2003
that this may be an important mechanism
leading to inflammatory cell accumulation
and activation during kidney disease. The
increase in MIF immunostaining in the
biopsy tissues is consistent with the
previous study done by Lan et al. (2000) in
which the upregulation of MIF mRNA and
protein expression was described in a
different cohort of GN patients.

This study has shown that in an
individual patient, the urine and renal MIF
correlated with the severity and activity of
GN (glomerular hypercellularity and
crescent formation, the degree of interstitial
damage, mononuclear cell infiltrate, and
loss of renal function). Therefore, the urine
MIF concentration may be useful in
monitoring patients for the degree of
disease activity. However, Brown et al.
(2002) found that urine MIF concentration
is not useful in identifying a specific type of
GN in an individual patient, although a high
urine MIF level does suggest a more severe
proliferative form of GN. Further studies
Macrophage Migration Inhibitory Factor in Systemic……..

are needed to assess how urine MIF excretion changes with time in individual patients. In particular, it is important to determine whether urine MIF could be an early indicator of a flare of disease activity.

In summary, this study found that the serum concentration of MIF is more likely to be elevated above the normal range in SLE, as in other inflammatory diseases. Also, urine MIF concentration is significantly increased in SLE World Health Organization class IV and correlated with the degree of renal dysfunction, histological damage, and leukocytic infiltration. Prospective studies of the association between MIF and disease activity using validated indices of disease activity, and including patients with active disease, are required.

References


لمعامل المثبط لانتقال الماكروفاج في مرض الذبابة الحمراء

وتمثيلها في الحالات المصحوبة بالتهاب الكلى

نحلة خطاب 1, نهاد النشار 1, نيفين بدر 2, حنان فهمي 2, راجيا فهمي 2, نهلا عوض 2

1- المركز القومي لبحوث و تكنولوجيا الإشعاع 2- كلية الطب, جامعة عين شمس - القاهرة - مصر

أهداف البحث

تهدف من هذه الدراسة إلى التوصل إلى احتمال تضمن المعامل المثبط لانتقال الماكروفاج في مرض الذبابة الحمراء وعلاقاته بجرعة الكورتيزون وإضاها قياس مستوى المعامل المثبط لانتقال الماكروفاج في مصل وبول مرض الذبابة الحمراء. كما استهدفت الدراسة تحديد تمثيل المعامل المثبط لانتقال الماكروفاج الكلوي في الحالات المصحوبة بالتهاب الكلى.

طرق البحث

تم قياس تركيز المعامل المثبط لانتقال الماكروفاج في المصل والبول بالطريقة الإنزييمية المناعية في عشر مريضات الذبابة الحمراء والصحوبة بالتهاب كلوي مصاحب للمرض من الدرجة الثانية، الثالثة والرابعة حسب درجات منظمة الصحة العالمية وفي عشرة السيدات الأصحاء من نفس المتوسط العمرى. وقد خضعت كل المريضات إلى فحص إكلينيكي مفصل وتحليل معهولة. وقد تم تقييم تمثيل المعامل المثبط لانتقال الماكروفاج الكلوي عن طريق الصبغة المناعية لعينات الأنسجة.

نتائج البحث

أظهرت النتائج ارتباط موجب بين تركيز المعامل المثبط لانتقال الماكروفاج في المصل وتلف مرض الذبابة الحمراء، فقد كانت معراثات التلف لمرض أكثر من المعامل المناعي لمرض الماكروفاج أعلى من المعدل المتوسط الطبيعي في المصل. كما لوحظ أن تركيز المعامل المثبط لانتقال الماكروفاج في المصل كان أعلى بدارة إحصائية في المريضات ذات درجة معامل تلف ≥ 3. كما وجد أن المعامل المثبط لانتقال الماكروفاج في المصل كان مرتبط ارتباطاً موجباً بجرعة الكورتيزون المستخدمة. كما وجد أن المعامل المثبط لانتقال الماكروفاج في المصل كان مرتبئ ارتباطاً سلباً مع تركيز الكورتيزون في المصل بشكل غير معتمد على تلف المرض أو جرعة الكورتيزون.

كما أوضحت النتائج أيضاً أن تركيز المعامل المثبط لانتقال الماكروفاج في البول كان أعلى بمعدل 2.98 مرة في مريضات الذبابة الحمراء عن في الأصحاء. كما أوضحت الصبغة المناعية لعينات الأنسجة عن زيادة لها دالة إحصائية في تمثيل المعامل المثبط لانتقال
الماكروفاج في مرضى الذبابة الحمراء والمصحوبة بالتهاب الكلى وكان هذا التمثيل مصحوب بارتشاح للمكروفا ج وخلايا (ت).

كما وجد ارتباط ذو دلالة إحصائية بين مستوى المعامل المثبط لانتشار الماكرافاج في البول وتمثيله في الكلى ولكن ليس مع مستواه في المصل مما يوضح أن مصدر المعامل المثبط لانتشار الماكرافاج في البول هو الكلى.

كما ارتبط مستوى المعامل المثبط لانتشار الماكرافاج في البول مع درجة تأثر الكلى، التلف الهستولوجي وارتشاح خلايا الدم البيضاء ولكن ليس مع كمية البروتين في البول.

الاستنتاج

يستند من الدراسة أن مستوى المعامل المثبط لانتشار الماكرافاج في المصل أعلى في مرضى الذبابة الحمراء عنه في الأصحاء كما أن مستواه في البول أعلى إحصائياً في مرضى الذبابة الحمراء من الدرجة الرابعة من درجات منظمة الصحة العالمية ويتناوب مستواه مع درجة إصابة الكلى. وعلى ذلك يعكس مستوى المعامل المثبط لانتشار الماكرافاج في البول درجة تمثيل المعامل في الكلى.