Original Research Article

Study of Serum Midkine Level in Patients with Rheumatoid Arthritis

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A B S T R A C T

Rheumatoid arthritis (RA) is a systemic autoimmune disease of unknown etiology. Midkine (MK) is a pleiotropic growth factor prominently expressed during embryogenesis but down-regulated to negligible levels in healthy adults. MK is involved in cell proliferation, differentiation, migration, and survival. The present study aimed to investigate the role of serum midkine level in rheumatoid arthritis (RA) patients and to analyze the relationship between the serum MK level and disease activity. Blood samples were collected from 60 patients with rheumatoid arthritis and twenty healthy individuals served as control group. Serum level of Midkine was measured by Enzyme Linked Immunosorbent Assay (ELISA). Other markers including Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), rheumatoid factor (RF), and Anti-CCP were estimated. Serum Midkine level was significantly higher in active RA patients than in inactive RA patients and healthy controls. It correlated positively with DAS-28 score in RA patients. ROC curve analysis revealed that the diagnostic efficacy of midkine was of 0.843 and the prediction power of RA activity was of 0.825. MK was significantly increased in serum of RA patients, and its level was correlated with several clinical markers of RA and DAS-28 score. So, the serum MK level could be a marker of RA disease activity.

Introduction

Rheumatoid arthritis (RA) is characterized by chronic inflammation of multiple joints. The synovial tissue of RA patients features proliferation of synoviocytes, accumulation of inflammatory cells (including lymphocytes and macrophages), production of inflammatory mediators, and angiogenesis (1). Midkine (MK) is a pleiotropic growth factor prominently expressed during embryogenesis but down-regulated to negligible levels in healthy adults. MK is involved in cell proliferation, differentiation, migration, and survival (2).

Many published studies have demonstrated striking MK overexpression compared with healthy controls in various pathologies, including ischemia, inflammation, autoimmunity and, most notably, in many cancers (3). MK expression is detectable in biopsies of diseased, but not healthy, tissues. Significantly, because it is a soluble cytokine,

elevated MK is readily apparent in the blood and other body fluids such as urine and cerebrospinal fluid (CSF), making MK a relatively convenient, accessible, non-invasive and inexpensive biomarker for population screening and early disease detection (4).

MK expression plays a significant role in inflammatory and autoimmune diseases, including in disease models of rheumatoid arthritis (RA) (5)and multiple sclerosis (MS) (6).Limited human data show that MK is elevated in the synovial fluid and sera of RA patients. Further basic studies investigating MK elevation in human subjects are required to determine whether MK offers utility as a biomarker in RA (4). This study aimed to investigate the role of serum midkine level in rheumatoid arthritis (RA) patients and to analyze the relationship between the serum MK level and disease activity.

Materials and Methods

The present study was conducted on 60 patients with rheumatoid arthritis (Group 1) and twenty healthy individuals served as control group (Group 2). Patients were selected from outpatient clinic and inpatients of Rheumatology unit, internal medicine department, Tanta University hospitals during the period from January 2018 to August 2018. This study was conducted in clinical pathology department, Tanta University Hospital. They were diagnosed according to the classification criteria of the American College of Rheumatology/ European League Against Rheumatism (7). They were further subdivided according to the disease activity based on Disease Activity Score 28 (DAS-28) into: Group 1A: Thirty patients with active rheumatoid arthritis. They were 6 males and 24 females with age ranged from 20-61 years. Group 1B: Thirty patients with inactive rheumatoid arthritis. They were 4 males and

26 females with age ranged from 23-64 years. The healthy controls were 3 males and 17 females with age ranged from 22-62 years. They were age and sex matched with RA patients. The study was approved by the ethical committee of faculty of medicine, Tanta University and written informed consents were obtained from all participants. Patients with malignant diseases, cardiac diseases, inflammatory diseases and auto immune diseases other than rheumatoid arthritis were excluded from this study.

All patients and controls were subjected to the following: Complete clinical examination, routine laboratory investigations including complete blood cell count (CBC),erythrocyte sedimentation rate (ESR) by Westergren tube method, C- reactive protein (CRP), and rheumatoid factor (RF) via immunoturbidimetric assay on automated chemistry analyzer; Konelab 60 I, Thermo Scientific, Vantaa, Finland, Anti- cyclic citrullinate peptide antibodies (anti-CCP) by electro-chemiluminescence immunoassay (ECLIA) on automated immunoassay analyzer, Cobas e411, Roche Diagnostics GmbH, Mannheim, Germany. The level of disease activity was assessed by DAS-28 and can be interpreted as inactive RA (DAS-28<2.6), and active RA disease (DAS-28 > 2.6)(8).

Immunoassay of serum MidKine level

Three ml blood were collected from RA patients and controls in a plain tube then centrifuged for 15 minutes at 3000 r.p.m for separation of serum, and then stored at -20C until assay of midkine level. The midkine level was estimated via enzyme linked immunosorbent assay (ELISA) (SUNRED., CHINA). The colorimetric detection was performed on a micro plate reader at 450 nm. The standard curve was drawn by plotting the mean absorbance obtained from each standard

against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis using linear graph paper. The concentration of the samples was determined directly from this standard curve.

Statistical analysis

The data collected were tabulated and analyzed by SPSS (statistical package for social science) version 22.0 on IBM compatible computer (SAS Institute, 2005).Chi-square test (χ2) was used to study association between two qualitative variables. ANOVA (f) test was used for comparison between three or more groups having quantitative variables. Kruskal-Wallis test was used for comparison between three or more groups not normally distributed having quantitative variables. The ROC (receiver operating characteristic) curves analysis was used to assess the diagnostic performance of midkine and the sensitivity and specificity were calculated. Youden's index was used to determine the optimal cut-off. P- value less than 0.5 is considered significant.

Results and Discussion

Demographic Characteristics of the studied groups

In this study, 60 RA patients were recruited and further divided into two groups; group1A (active RA) and group 1B (inactive RA), in addition to 20 healthy controls as group 2. No significant difference between the three groups regarding age and sex (Table 1).

Clinical and Laboratory Characteristics of the studied groups

The inflammatory markers such as ESR and CRP as well as autoimmune markers as RF and Anti-CCP levels were significantly higher in active RA patients than inactive RA patients and healthy controls. Moreover, serum Midkine level was significantly higher in active RA patients than inactive RA patients and healthy controls. As expected the DAS-28 score was significantly higher in active RA patients than inactive RA patients (Table 1).

Correlation between serum Midkine level and demographic, clinical and laboratory characteristics of the patients' groups

Pearson correlation analysis between serum midkine level with age, ESR, CRP, RF, Anti-CCP levels, and DAS-28 score in RA patients revealed that serum midkine level with ESR, RF, serum Anti-CCP, DAS-28 score levels in active RA patients and with ESR level and DAS-28 score in inactive RA patients (Table 2).

Performance characteristics of serum MidKine in the studied group

Roc curve analysis of the performance characteristics of the MidKine was performed. For RA diagnosis, Midkine had AUC of 0.843 whereas, serum anti-CCP had AUC of 0.833, and RF had AUC of 1.000 (Figure 1). At a cutoff value of >230pg/mL, midkine was of 91.0 % sensitivity and 65.0 % specificity in differentiating RA patients from healthy subjects. For prediction of active RA patients, midkine had AUC of 0.825 whereas, ESR had AUC of 0.959, and CRP had AUC of 0.984 (Figure 2). At a cutoff value of >310 pg/mL, midkine was of 83.0% sensitivity and 70.0% specificity in differentiating active from inactive RA patients.

Rheumatoid arthritis (RA) is a chronic inflammatory disease that affect from 0.5% to 1% of population, therefore it considered the most common chronic inflammatory autoimmune disease. Early diagnosis for RA

is very important and it is difficult because early presentation of RA patients may confuse with other inflammatory arthritis. Moreover, the classical clinical picture of RA tends to present after the progression of the disease. Additionally, treatment with anti-inflammatory drugs or corticosteroids may mask symptoms and signs (9).This study was designed to investigate the role of serum midkine level in rheumatoid arthritis (RA) patients and to analyze the relationship between the serum MK level and disease activity.

Table.1 Demographic, clinical, and laboratory characteristics of the studied groups

	Group 1A (n-30)	Group 1B (n=30)	Group2 (n=20)	P-value
Age (years)	43.27±10.03	41.93 ±10.39	44.2±9.89	0.729
Sex (Male/Female) n	6 / 24	4 / 26	3/17	0.771
ESR (mm)	81.27±17.78[ab]	14.87±6.20	7.95±2.48	**<0.001***
CRP (mg/l)	48.87±26.49[ab]	5.07±2.88	3.95±1.47	**<0.001***
RF (IU/ml)	99.47±22.21[ab]	27.13±16.27[a]	3.50±1.61	**<0.001***
Anti-CCP (U/ml)	90.67±46.09[ab]	45.17±24.68	26.15±24.65	**<0.001***
DAS score	4.72 ± 1.06	1.79 ± 0.52	----	**<0.001***
SerumMidkine (pg/ml)	458.33±157.92[ab]	301.67±73.02	236.5±63.10	**<0.001***

Table.2 Correlation of serum Midkine level with age, ESR, CRP, RF, Anti-CCP levels and DAS score in RA patients

	Midkine (pg/ml)			
	Active RA		**Inactive RA**	
	r	**P-value**	**R**	**P-value**
Age (Years)	0.014	0.533	0.001	0.853
ESR (mm/1h)	0.371	0.001*	0.253	0.005*
CRP (mg/dl)	0.048	0.246	0.012	0.578
RF (IU/ml)	0.430	0.001*	0.017	0.486
Anti-CCP (U/ml)	0.251	0.005*	0.098	0.092
DAS score	0.289	0.002*	0.178	0.020*

Fig.1 Roc curve analysis of the MidKine, Anti-CCP, RF levels between RA patients and healthy controls
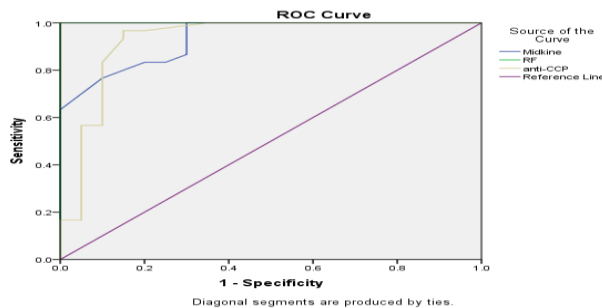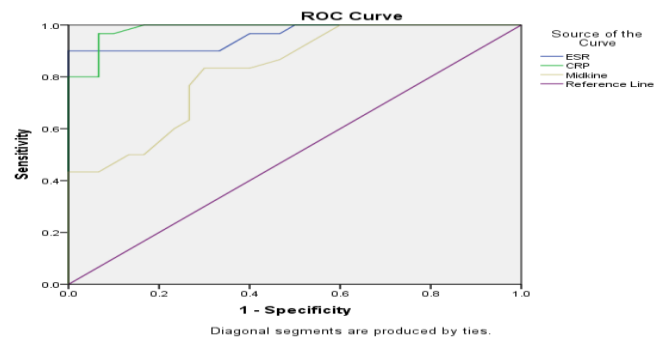
Fig.2 Roc curve analysis of the MidKine, CRP, ESR levels between active RA patients and inactive RA patients

ESR and CRP are considered as markers of inflammation which indicate the disease activity in RA patients. In the present study, ESR and CRP were high in active RA patients group compared to healthy controls group and inactive RA. Consistent with our results, study conducted by Ranganath *et al.,* (10)reported that ESR is one of laboratory indicators of the disease activity in RA patients. Wislowska and Jablonska, (11) and Massoud *et al.,* (12) found that CRP was elevated in patients with early RA.

In this study, serum RF and Anti-CCP levels were significantly higher in RA patients than healthy subjects. RF is the most sensitive laboratory serologic marker for the diagnosis of RA according to the American College of Rheumatology (ACR). However, the specificity of RF is relatively low because there is a 50% positive rate of RF in patients with other connective-tissue diseases, such as systemic lupus erythematosus (SLE), primary Sjögren syndrome, and dermatomyositis, with some infections; and in healthy elderly persons, which limits its diagnostic value (13). Anti-CCP antibodies have the power to predict the development of RA in patients with early and undifferentiated arthritis (14).The new criteria for diagnosis of RA have been introduced and anti-CCP, together with RF, is included therein (7).

As regard serum midkine level, the results of the present work showed that there was a significant elevation of serum midkine levels in active RA patients compared to inactive RA patients and healthy subjects. For RA diagnosis, midkine had AUC of 0.843. At a cutoff value of >230 pg/mL, midkine was of 91.0 % sensitivity and 65.0 % specificity in differentiating RA patients from healthy subjects. In agreement of the results of the present work, Shindo *et al.,* (1) showed that the serum MK level is elevated in RA patients and is correlated with clinical indexes of RA. Previous study has shown that the serum MK level is elevated in RA patients and is correlated with the RF titer (5).

In this study, serum midkine level correlated positively with DAS-28 score which indicates that the serum MK level could be a useful marker of RA disease activity. For prediction of active RA patients, Midkine had AUC of 0.825. At a cutoff value of >310 pg/mL, midkine was of 83.0% sensitivity and 70.0% specificity in differentiating active from inactive RA patients. Shindo *et al.*, (1)found that the serum MK level was correlated with DAS28-ESR and that MK tended to be decreased after treatment with an anti-TNF-a antibody.

MK has been implicated in a wide number of biological functions including e.g.

angiogenesis under hypoxic conditions (15), cell survival (16), and tumor growth(17). Multiple studies also suggested an important role for MK in several pathological inflammatory conditions (18). Accordingly, MK is significantly up regulated in inflamed synovial tissue during an active rheumatoid arthritis flare in contrast to synovial tissue of healthy patients (19).

MK was expressed in the RA synovial lining and synovial fluid, with minimal MK expression in OA synovium and synovial fluid (5)**.**This is further confirmed by findings in a mouse arthritis model, where leukocyte infiltration and joint damage are markedly attenuated in MK-/- mice (5).Thus, MK could be a potential therapeutic target in patients with RA.

Stimulation with MK enhanced the production of IL-6, IL-8, and CCL2 by cultured RSFs. IL-8 is a chemokine (C-X-C motif) ligand 8 (CXCL8), which induces blood vessel formation and angiogenesis and also exhibits a chemoattractant activity for neutrophils and dendritic cells (20). CCL2 is thought to induce migration of monocytes into inflamed RA synovial tissue (21). Moreover, it was reported that MK induces neutrophil migration (19), so MK could both directly and indirectly promote inflammatory cell accumulation in RA synovial tissue and synovial fluid. It has been reported that MK enhances endothelial cell proliferation and vascular growth (22) and also up-regulates the differentiation of osteoclasts in vitro (5). Therefore, MK may contribute to angiogenesis and bone destruction in the synovial tissues in RA, as well as stimulating RSFs.

In conclusion, MK was significantly increased in serum of RA patients, and its level was correlated with several clinical markers of RA and DAS-28 score. So, the serum MK level could be a marker of RA disease activity. However, large number of cases and follow up the patients over time is recommended to determine prognostic value of serum midkine level in RA patients. Also, it is recommended to study serum midkine level in comparison with synovial midkine level and its correlation with disease severity.

Disclosure

The authors report no conflicts of interest.

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Author Contributions

All authors contributed equally in the writing of this manuscript.

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