Diagnostic value of the serum Midkine in patients with rheumatoid arthritis

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ABSTRACT

Early diagnosis and detection of rheumatoid arthritis (RA) activity which is a potential therapeutic target, depends mainly on clinical presentation. However, laboratory tests may contribute to diagnosis and disease activity assessment of RA. This study aims to evaluate the accuracy of serum Midkine as serological marker for RA diagnosis and its activity detection. All patients with RA were recruited during the period from January 2016 to August 2018 in addition to healthy subjects as control. Serum Midkine level was estimated using enzyme immunoassay. The accuracy was determined for serum Midkine against the used American College of Rheumatology/European League Against Rheumatism 2010 classification criteria for RA diagnosis and disease activity score derivative for 28 joints-erythrocyte sedimentation rate (ESR) score for assessment of RA disease activity. A total of 211 of patients with RA (group I) were enrolled in this study with 112 healthy subjects (group II). Patients with RA were divided into two subgroups according to the disease activity; patients with active RA (group IA) and RA in remission (group IB). We detected that the area under curve (AUC) of serum Midkine level (AUC=0.851) was significantly lower than that of rheumatoid factor IgM and anti-cyclic citrullinated peptide IgG for RA diagnosis. However, Midkine presents a significantly higher diagnostic accuracy (AUC=0.939) in detecting RA activity than that offered by C reactive protein (CRP) or ESR. Our study suggested that serum Midkine is a potential serological marker for detection of active inflammatory state with higher diagnostic accuracy than other inflammatory markers as CRP or ESR. Therefore, it can be used as an inflammatory marker for detection of disease activity rather than diagnosis of RA.

Significance of this study

What is already known about this subject?
- The diagnosis and detection of rheumatoid arthritis (RA) activity are based mainly on assessment of the clinical features.
- Several laboratory tests such as C reactive protein (CRP), erythrocyte sedimentation rate (ESR), anti-cyclic citrullinated peptide (anti-CCP), rheumatoid factor (RF) are now involved in the diagnostic criteria of RA and in the disease activity assessment scores.

What are the new findings?
- Herein, we conduct this study to evaluate the accuracy of serum Midkine as serological marker for the RA diagnosis and its activity detection.
- We detected a lower accuracy of the serum Midkine (area under curve (AUC)=0.851) for the diagnosis of RA when compared with either RF or anti-CCP accuracy.
- Regarding RA activity detection, serum Midkine has a significantly higher diagnostic accuracy (AUC=0.939) than that of CRP or ESR.

How might these results change the focus of research or clinical practice?
- Serum Midkine can be used as a reliable marker for the detection of active inflammatory conditions as active RA and suggested to be integrated into a new score for assessment of RA disease activity.

INTRODUCTION

Rheumatoid arthritis (RA) is the most globally prevalent inflammatory arthropathy that affects about 0.5%–1% of the world's population with a mean annual incidence of 0.02%–0.05%.[1] The disease affects all races worldwide with a female predominance.[2] It is a chronic heterogeneous autoimmune disease characterized by articular and extra-articular systemic manifestations.[3] The articular manifestation is due to the presence of long-standing chronic inflammation of multiple joints with proliferation of synoviocytes, accumulation of inflammatory cells (including lymphocytes and macrophages), production of inflammatory mediators, and angiogenesis. This results in symmetric polyarthritis with progressive joint, cartilage and bone damage leading to joint deformity,[4] disability,[5] poor life quality,[6] and premature mortality.[7]

Several laboratory markers as C reactive protein (CRP), erythrocyte sedimentation rate (ESR), anti-cyclic citrullinated peptide (anti-CCP), rheumatoid factor (RF) had been used and integrated with the clinical signs as synovitis in the currently used diagnostic criteria of the American College of Rheumatology/

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European League Against Rheumatism 2010 (ACR/EULAR 2010) for RA.[8] Also, the RA disease activity is assessed by multiple scoring methods[9] with the disease activity score derivative for 28 joints (DAS28) which is estimated mathematically from the tender, swollen joint count on 28 joints with CRP or ESR levels and global health evaluations of the degree of disease activity,[10] has been identified by the ACR as the most practically feasible because it is clinically easy, of high accuracy, sensitivity, discrimination capability between the grades of disease activity and has remission criteria.[9]

However, these laboratory markers were found neither sensitive nor specific. Also, early RA appears to be presented with incomplete or even non-classical clinical features.[11] Thus, new markers with high diagnostic accuracy are needed for the diagnosis and detection of RA activity or even to be integrated within the diagnostic and activity criteria of RA.

Midkine is a 13 kDa heparin-binding growth factor induced mainly by retinoic acid.[12] It has pleiotropic activities as it enhances cell proliferation, differentiation, survival, and migration.[13] It is also involved in angiogenesis and oncogenesis.[14 15] In addition, functional evidence has supported its potential role in inflammation.[13] It exerts this role via dual mechanisms; the first is the chemotaxis of neutrophils, macrophages and suppression of regulatory T cells expansion[16 17] and the second via its fibrinolytic activity which degrades the basement membranes, thus facilitates the infiltration of leukocytes from the blood to the tissues. These mechanisms explain the pathological significance of the Midkine in early stages of tissue inflammation.[16]

Functionally, the development of antibody-induced RA was inhibited in Midkine-deficient mice.[17 18] Clinically, several studies have detected the increased serum Midkine level in different inflammatory disorders including RA, osteoarthritis, systemic lupus erythematosus, ulcerative colitis, and Crohn's disease.[18–23] Significantly, because it is a soluble cytokine, Midkine appears rapidly in the blood and other body fluids whenever elevated, making Midkine a relatively appropriate, accessible, non-invasive and affordable biomarker for screening, and early disease detection.[24] Specifically, few studies suggested that Midkine is elevated in the serum and synovial fluid of patients with RA. Hence, further studies are required to evaluate the utility of Midkine as a biomarker in RA.

As the Midkine seems to be a mediator implicated in the pathogenesis of several inflammatory conditions and increased serum Midkine level was detected in many inflammatory disorders, then the serum Midkine has been suggested as an inflammatory marker for diagnosis of RA and detection of the inflammatory activity inpatients with RA. This study aimed to validate the accuracy of serum Midkine as a serological marker for the diagnosis of RA and even more to assess its performance regarding the detection of RA disease activity.

SUBJECTS AND METHODS

Study design

This was a case-control retrospective study conducted at Tanta university hospitals and adopted to assess the diagnostic accuracy of Midkine as a serological marker in patients with RA.

Subjects' recruitment

In this study, all patients with RA previously diagnosed according to the classification criteria of the ACR/EULAR 2010[8] and achieving at least score 6 out of 10 points were eligible to participate. The recruitment was designed in convenience series way. Accordingly, we screened 60 patients with RA who were admitted to the inpatient of rheumatology unit at the internal medicine department, Tanta University hospital and 216 patients with RA at the outpatient clinic on their follow-up visit during the period from January 1, 2016 till August 31, 2018.

Out of the previously screened patients with RA, 34 patients were excluded from the study due to malignant diseases, cardiac diseases, and other inflammatory diseases. Thirteen patients declined the participation and 18 patients did not attend the sampling visit. Accordingly as illustrated in the flow chart (figure 1), 211 patients with RA were included in addition to 112 healthy subjects serving as a study control.

Subjects' categorization

All the included patients with RA were assigned to the first group (group I, or patients with RA) which further divided based on the status of their disease activity using DAS28-ESR score[10] into two subgroups; group IA, patients with active RA having DAS28-ESR score >2.6 and group IB, patients with RA in remission with DAS28-ESR score<=2.6. The healthy subjects were assigned to the second group (group II, or healthy control).

Clinical assessment

The medical records of each of the included patients were reviewed and all the patients were asked about the history of their disease status. Full clinical examination with assessment of disease activity by DAS28-ESR was performed using four variables; tender and swollen joints count out of 28 joints, ESR level and patient global health assessment of the disease activity.

Sampling

In the sampling visit, seven millimeters of venous blood were collected from each subject by use of disposable sterile plastic syringe under sterile conditions. Each sample was fractionated as following; 2 mL were placed in the sodium citrate tube for ESR estimation followed by the plain tube for estimation of CRP, RF IgM, anti-CCP IgG and serum Midkine levels. The serum was separated from the blood collected in a plain tube after centrifugation at 3000 RPM for 15 minutes then was divided into two aliquots: one for immediate estimation of CRP, RF IgM, and anti-CCP IgG levels, and the other aliquot was stored at −20°C until assay of Midkine level.

Laboratory analysis

The ESR was measured using Westergren's method. The CRP and RF IgM levels were estimated via immunoturbidimetric assay on automated chemistry analyzer; Konelab 60 I, Thermo Scientific, Vantaa, Finland, using kits purchased from Thermo Fisher Scientific, Vantaa, Finland, Catalog no: CRP-Plus, 981794 & RF-2, 981920. The intra-assay coefficient of variations (CV) were; 1.2%, 0.8% whereas;

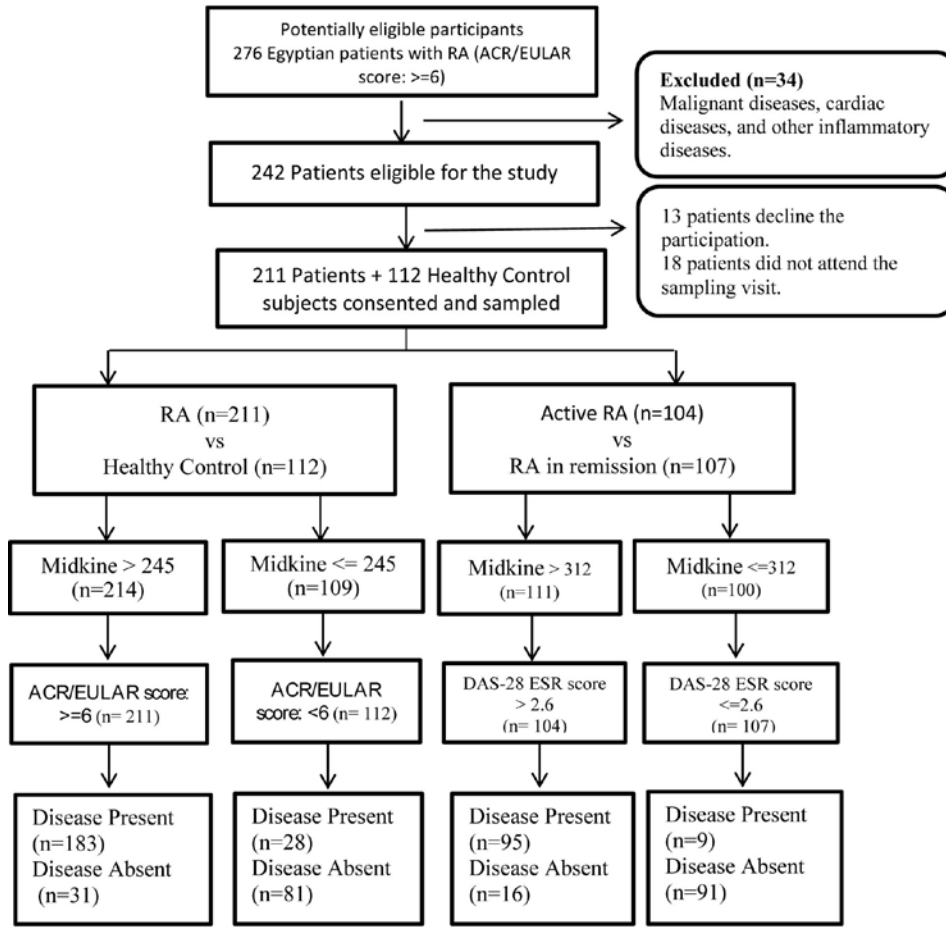

Figure 1 Flow chart of the study design and the studied groups. ACR/EULAR, American College of Rheumatology/European League Against Rheumatism; DAS28, disease activity score derivative for 28 joints; RA, rheumatoid arthritis.

interassay CVs were 0.4%, 2.6% for CRP and RF IgM respectively. The anti-CCP IgG level was measured by electro-chemiluminescence immunoassay on automated immunoassay analyzer, Cobas e411, Roche Diagnostics GmbH, Mannheim, Germany, using kit purchased from Roche Diagnostics GmbH, Catalog no: 05031656. The sensitivity was 67.4%, specificity of 97%, interassay CV of 2.3%, and intra-assay CV of 4.5%.

Enzyme immunoassay of serum Midkine level

The serum Midkine level was estimated by sandwich ELISA technology using human Midkine ELISA kit provided by Wuhan Fine Biological Technology Co., Wuhan, Hubei, China, catalog no: EH0229. The reagents were prepared according to the manufacturer package insert data and serial dilutions of the standard with the provided dilution buffer from 15.652 to 1000 pg/mL were obtained. The assay was performed onto a 96-well microtiter plate following the procedure steps in the package insert with colorimetric detection using Tecan Spectra II Microplate Reader (Männedorf, Switzerland). The logit-log standard curve was displayed and from which the sample concentrations were calculated. The intra-assay and interassay CVs were less than 8% and 10% respectively.

Statistics

In this study, we use the student t-test for the comparison between the studied groups regarding the numerical normally distributed parameters, Mann-Whitney U test regarding the non-normally distributed parameters and X^2 test regarding the nominal data. The serum Midkine level was correlated with different clinical and laboratory data using Pearson correlation and multivariate analysis was performed using the multiple logistic regressions for all significant variables in the univariate analysis. ROC curve analysis was performed to assess the performance specifications of the serum Midkine between the studied groups. P values less than 0.05 were considered significant. All statistical analysis was performed via the SPSS V.22 and medCalc softwares.

RESULTS

Baseline characteristics of the studied groups

In our study, 211 patients with RA (group I) were recruited according to the eligibility criteria in addition to 112 healthy subjects (group II) as illustrated in the flowchart (figure 1). The patients with RA were further assigned to two subgroups based on their disease activity status. The basic demographic, clinical and laboratory features for all the study groups and subgroups are demonstrated in table 1.

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

Characteristic	Group I (RA) (n=211)	Group II (HC) (n=112)	P value	Group IA (Active RA) (n=104)	Group IB (RA in remission) (n=107)	P value
Age (y)	42.68±8.86	44.59±9.87	0.078	43.63±9.48	41.77±8.14	0.128
Gender n (%)						
Male	41 (19.4%)	19 (17.0%)	0.854	19 (18.3%)	22 (20.6%)	0.721
Female	170 (80.6%)	93 (83.0%)		85 (81.7%)	85 (79.4%)	
Tender joints (n)	3.0 (0–9)	—-	N/A	5.0 (3–9)	0.0 (0–3)	**<0.001***
Swollen joints (n)	2.0 (0–8)	----	N/A	4.0 (2–8)	0.0 (0–2)	**<0.001***
CRP (mg/L)	18.0 (1–96)	5.5 (1–18)	**<0.001***	26.0 (6–96)	12.0 (1–48)	**<0.001***
ESR (mm/h)	35.0 (5–98)	14.0 (4–55)	**<0.001***	52.0 (12–98)	28.0 (5–40)	**<0.001***
RF (IU/mL)	63.0 (8–230)	10.0 (1–22)	**<0.001***	65.0 (8–230)	63.0 (9–212)	0.827
Anti-CCP (U/mL)	80.0 (10–212)	15.0 (3–38)	**<0.001***	85.0 (10–212)	80.0 (10–145)	**0.037***
DAS28 (ESR) score	3.74+1.26	----	N/A	4.99±0.35	2.33±0.10	**<0.001***

Notes. Data are expressed as mean±SD or median and IQR.
*P<0.05 significant.
Anti-CCP, anti-cyclic citrullinated peptide; CRP, C reactive protein; DAS, disease activity score; ESR, erythrocyte sedimentation rate; HC, healthy control; RA, rheumatoid arthritis; RF, rheumatoid factor.

The group IA included 104 patients with active RA, aged 43.63±9.48 years, 19 males and 85 females, with a DAS28-ESR score of 4.99±0.35, a median RF IgM level of 65.0 IU/mL and a median anti-CCP IgG level of 85.0 U/mL. The group IB included 107 patients with RA in remission, aged 41.77±8.14 years, 22 males and 85 females, having a DAS28-ESR score of 2.33±0.10 with a median RF IgM level of 63.0 IU/ml and a median anti-CCP IgG level of 80.0 U/mL. The control group II included 112 healthy subjects, aged 44.59±9.87, 19 males and 93 females with a median RF IgM level of 10.0 IU/mL and a median anti-CCP IgG level of 15.0 U/mL.

Serum Midkine distribution level between the studied groups

The mean serum Midkine level was 396.87±177.22 with a range (200–850 pg/mL) in group I whereas, in group II, its mean value was 220.58±68.70 with a range (118–350 pg/mL). Furthermore, in group IA serum Midkine had a mean value of 520.80±171.77 with a range (280.0–850.0 pg/mL) and in group IB, its mean value was 276.41±61.91 with a range (200.0–450.0 pg/mL). There was a significant elevation of serum Midkine levels in patients with RA (group I) when compared with healthy control (group II) (p<0.001) and in patients with active RA (group IA) compared with patients with RA in remission (group IB) (p<0.001) (table 2 & figure 2).

Table 2 Serum Midkine distribution levels between the studied groups

Study groups	Serum Midkine level (pg/mL)	P value
Group I (RA) (n=211)	396.87±177.22	**<0.001***
Group II (HC) (n=112)	220.58±68.70	
Group IA (Active RA) (n=104)	520.80±171.77	**<0.001***
Group IB (RA in remission) (n=107)	276.41±61.91	

Notes. Data are expressed as mean±SD.
*P<0.05 significant.
HC, healthy control; RA, rheumatoid arthritis.

Clinical and biochemical effects on the serum Midkine level

Univariate analysis using Pearson correlation revealed a significant positive correlation of serum Midkine level with the tender, swollen joint count, ESR, serum anti-CCP IgG, RF IgM, CRP levels and DAS28-ESR score irrespective of age and gender of the patients with RA group. However multivariate logistic regression analysis revealed that serum Midkine level was independently related with the tender, swollen joint count, ESR level and DAS28-ESR score irrespective of anti-CCP IgG, RF IgM and CRP levels (table 3).

Serum Midkine performance characteristics

The diagnostic accuracy of the serum Midkine in differentiating between the studied groups was assessed by the ROC curve analysis. Using the classification criteria of the ACR/EULAR 2010, area under curve (AUC) of the serum Midkine was estimated as 0.851 with a sensitivity of 86.3%, a specificity of 72.3%, a positive predictive value (PPV) of 85.5% and a negative predictive value (NPV) of 74.3% at a cut-off value >245 pg/mL for differentiating the control

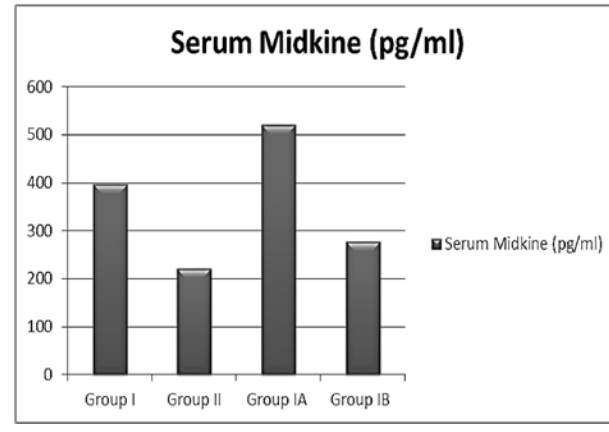

Figure 2 Serum Midkine distribution levels between the studied groups.

Table 3 Univariate and multivariate logistic regression analysis of the serum Midkine level with clinical and biochemical characteristics of patients with RA

	Patients with RA (n=211) Serum Midkine level (pg/mL)				
	Univariate analysis		Multivariate logistic regression analysis		
Characteristic	R	P value	Standardized Beta	T	P value
Age (y)	0.034	0.313	---	---	---
Gender	0.088	0.101	---	---	---
Tender joints (n)	0.835	**<0.001***	1.154	10.809	**<0.001***
Swollen joints (n)	0.859	**<0.001***	1.177	11.703	**<0.001***
CRP (mg/L)	0.269	**<0.001***	–0.023	–0.623	0.534
ESR (mm/h)	0.485	**<0.001***	0.826	11.002	**<0.001***
RF IgM (IU/mL)	0.193	**0.002***	–0.019	–0.629	0.530
Anti-CCP IgG (U/mL)	0.142	**0.020***	0.014	0.496	0.621
DAS28 ESR score	0.779	**<0.001***	1.936	11.658	**<0.001***

*P<0.05 significant.
Anti-CCP, anti-cyclic citrullinated peptide; CRP, C reactive protein; DAS, disease activity score; ESR, erythrocyte sedimentation rate; RA, rheumatoid arthritis; RF, rheumatoid factor.

subjects from the patients with RA (table 4, figure 3A). Also, on differentiating patients with active RA from patients with RA in remission against the DAS28-ESR score, AUC of the serum Midkine was estimated as 0.939 with a sensitivity of 91.3%, a specificity of 85.0%, PPV of 85.6% and NPV of 91.0% at a cut-off value >312.0 pg/mL (table 4, figure 3B).

Serum midkine accuracy against other RA diagnostic and activity laboratory variables

For diagnosis of RA, the diagnostic accuracy of Midkine (AUC=0.851) was significantly lower than that for anti-CCP (AUC=0.928, p=0.003) and that for RF (AUC=0.901) with no significance (p=0.065) (table 5 and figure 3A) whereas, for differentiating the active RA from RA in remission, the accuracy of serum Midkine (AUC=0.939) was significantly higher than that for CRP (AUC=0.758, p<0.001) and ESR (AUC=0.859, p=0.004) (table 5 and figure 3B).

DISCUSSION

RA is a systemic autoimmune disorder characterized by chronic joint inflammation which results in progressive joint damage and dysfunction.[25] Despite, the diagnosis being based mainly on clinical features; laboratory tests may have a major role in the diagnosis and assessment of RA disease activity. Functional evidences had linked the Midkine in the pathogenesis of many inflammatory and autoimmune diseases. However, few studies had evaluated the diagnostic value of Midkine in relation to the RA. To our knowledge, this is the second study to evaluate the diagnostic value of serum Midkine level in patients with RA and the first to investigate the diagnostic efficacy of serum Midkine as a marker of active inflammation in relation to the RA disease activity.

In this study, we observed that the serum Midkine level was significantly higher in patients with RA than in healthy subjects (p<0.001). Also, we found that patients with active RA exhibit a significant elevation of serum Midkine level when compared with patients with RA in remission (p<0.001). Accordingly, we suggest that serum Midkine may be useful in the diagnosis of RA and to detect the disease activity. In agreement with our results, a study conducted by Shindo *et al*,[21] detected a high level of serum Midkine in patients with RA and the overexpression of Midkine protein and mRNA in RA synovial tissues as well as the cultured rheumatoid synovial fibroblasts (RSFs). Previous functional studies detected that Midkine is significantly upregulated in inflamed synovial tissue during an active RA flare in contrast to synovial tissue of healthy patients.[16] This is further confirmed by findings in a mouse arthritis model, where leucocyte infiltration and joint destruction are markedly inhibited in Midkine deficient mice. Also, Midkine was found to be highly expressed in the RA synovium whereas, minimally expressed in osteoarthritis synovium.[18]

In our study, multivariate logistic regression analysis revealed that serum Midkine level was related independently with DAS28-ESR score, ESR irrespective of serum anti-CCP IgG, RF IgM, and CRP levels. In agreement with our results, Shindo *et al*,[21] found that the serum Midkine level was correlated with DAS28-ESR and tends to decrease after treatment with an anti-tumor necrosis

Table 4 ROC curve analysis of the serum Midkine performance characteristics between the studied groups:

Serum Midkine level (pg/ml)	AUC	P value	95% CI	Cut-off	Sensitivity	Specificity	PPV	NPV
Groups:								
Group I vs Group II	0.851	**<0.001***	0.808 to 0.888	**>245.0**	86.3	72.3	85.5	74.3
Group IA vs Group IB	0.939	**<0.001***	0.898 to 0.968	**>312.0**	91.3	85.0	85.6	91.0

*P<0.05 significant.
AUC, area under curve; NPV, negative predictive value; PPV, positive predictive value.

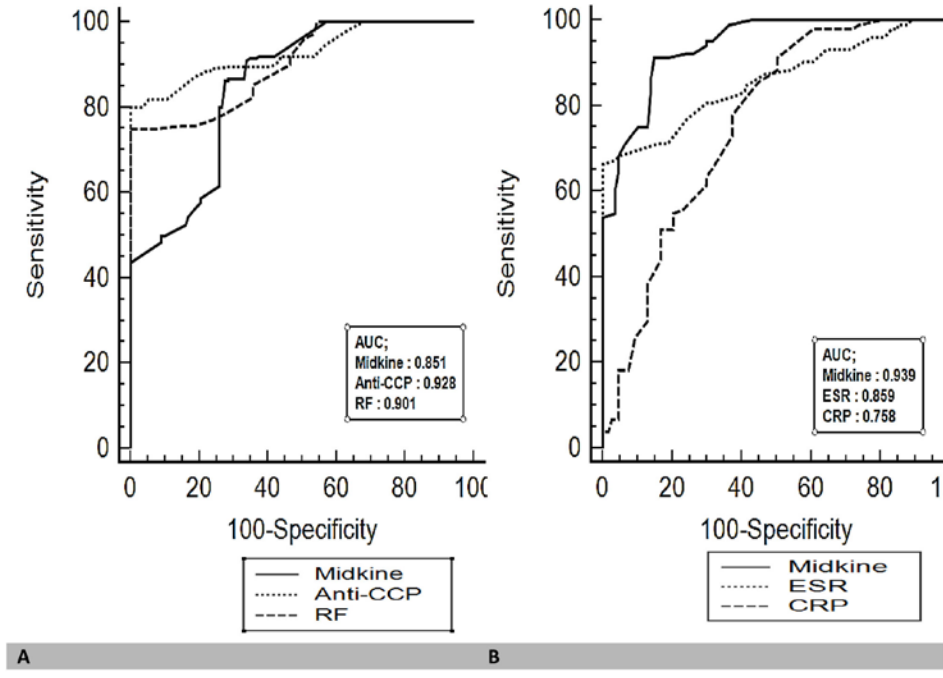

Figure 3 ROC curve analysis of the performance characteristics of serum Midkine, and other laboratory variables between the studied groups: (A) patients with RA (Group I) vs Healthy Control subjects (Group II) (B) patients with active RA (Group IA) vs patients with RA in remission (Group IB). Anti-CCP, anti-cyclic citrullinated peptide; AUC, area under curve; CRP, C reactive protein; ESR, erythrocyte sedimentation rate; RF, rheumatoid factor.

factor alpha (anti-TNF-α) antibody. Therefore, these findings suggest that serum Midkine level could be a useful marker for RA disease activity. However, contradictory to our results, the RF high titer was found to be correlated with the serum Midkine in several studies that suggest its relation with the poor prognosis of RA.[18 21 26]

Growing evidences suggested that Midkine contributed to the inflammation process of the synovial tissues of patients with RA via promoting the inflammatory cells accumulation in synovial tissue and synovial fluid either directly through stimulating the neutrophil migration[16] or indirectly via enhancing the production of interleukin-6 (IL-6), IL-8, and chemokine (C-C motif) ligand 2 (CCL2) by RSFs. IL-6 was found to regulate immune cell activation.[21] IL-8 induces angiogenesis and also exhibits a chemoattractant activity for neutrophils and dendritic cell.[27] CCL2 is thought to induce migration of monocytes into inflamed RA synovial tissue.[28] Also, Midkine has been reported to enhance endothelial cell proliferation[29] and osteoclasts differentiation in vitro[18] which promote the angiogenesis and bone destruction in the synovial tissues of patients with RA.

Despite, the diagnosis of RA based mainly on the clinical manifestations. The laboratory markers may be suggested to contribute and integrated with the clinical features to the diagnosis of RA as well as detection of disease activity. In 1987, the ACR defined RF as the only serologic marker for the diagnosis of RA due to its high sensitivity (70%). However, the specificity of RF is relatively low because there is a high positive rate of RF in patients with other connective-tissue diseases, viral infections, tumors and in healthy elderly persons, which limits its diagnostic value.[30 31]

Therefore, it is necessary to search for other laboratory diagnostic markers with high diagnostic accuracy. Many studies have focused on the diagnostic value of the anti-CCP antibody in RF and detected its high specificity (90%)[32 33] which favors the early diagnosis.[34] Therefore, anti-CCP, together with RF were included within the 2010 ACR/EULAR criteria for diagnosis of RA.[8] The result of the present work showed that the

Table 5 Comparison of the serum Midkine performance characteristics with other laboratory variables between the studied groups

	Group I vs Group II				Group IA vs Group IB			
		Serum Midkine (pg/mL)				Serum Midkine (pg/mL)		
	AUC	AUC difference	95% CI	P value	AUC	AUC difference	95% CI	P value
RF IgM (IU/mL)	0.901	0.049	−0.003 to 0.102	0.065	0.509	0.431	0.346 to 0.515	**<0.001***
Anti-CCP IgG (U/mL)	0.928	0.067	0.0249 to 0.128	**0.003***	0.583	0.356	0.270 to 0.442	**<0.001***
ESR (mm/h)	0.809	0.043	−0.0181 to 0.103	0.169	0.859	0.081	0.026 to 0.136	**0.004***
CRP (mg/L)	0.838	0.013	−0.0468 to 0.0728	0.426	0.758	0.181	0.111 to 0.251	**<0.001***

*P<0.05 significant.
Anti-CCP, anti-cyclic citrullinated peptide; AUC, area under curve; CRP, C reactive protein; ESR, erythrocyte sedimentation rate; RF, rheumatoid factor.

diagnostic accuracy of serum Midkine for RA detection was found to be significantly lower than that of the anti-CCP IgG or RF IgM which limit its value as a diagnostic marker for RA relative to the already used RF or anti-CCP.

ESR and CRP were used in the DAS score as a biomarker for the RA activity. Several studies had suggestedelevated levels of ESR or CRP[35–37] in the active stage of RA whereas, others suggested that ESR or CRP often do not correlate with disease activity in a large cohort of United States registry of patients with RA.[38] In our study, the diagnostic accuracy of serum Midkine on detecting the active RA was observed to be at a satisfactory level (AUC=0.939) with a sensitivity of 91.3% and a specificity of 85.0% at a cut-off value >312 pg/mL which is significantly higher than that of CRP and ESR. These findings support the pivotal role of serum Midkine as an inflammatory marker in the detection of active inflammation process as in patients with active RA. Therefore, we suggest the higher efficacy of serum Midkine as a marker for RA activity relative to the already used laboratory markers.

In conclusion, Midkine was significantly increased in serum of patients with RA, and its level was correlated with several clinical and biochemical markers of RA. The accuracy of Midkine is lower than that for RF or Anti-CCP for diagnosis of RA, however, its accuracy was satisfactory and higher than that of CRP and ESR regarding the detection of disease activity. Taking together these data with the ease of serum Midkine measuring, we suggest that serum Midkine could be a useful applicable marker for the detection of active inflammatory conditions as active RA with a greater accuracy than other routine inflammatory markers as CRP and ESR.

Limitations of the study

The current study is limited as it only defines the associations of the Midkine with RA not the etiology or pathogenesis. However the strength of the study is being the first to assess the role of serum Midkine in active inflammation and to compare the diagnostic accuracy of the serum Midkine against the DAS score for RA activity detection.

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Competing interests None declared.

Patient consent for publication Obtained.

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