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Research article

Study of the Diagnostic Value of Anti MCV and MMP3 Biomarkers in Serum and Synovial Fluid of Rheumatoid Arthritis Patients

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ABSTRACT

Background: Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by persistent joint inflammation, damage and loss of function.Osteoarthritis (OA) is a common progressive degenerative disease that affects the joint cartilage, subchondral bone, synovial and joint capsule resulting in disability. Early diagnosis of RA is an important challenge for clinical rheumatologists as it leads to a better disease outcome. Anti-mutated citrullinated vimentin(Anti-MCV) antibodies are member of ACPAs family, that result due to antibody production against antigens produced from the mutant citrullinated vimentin. The Matrix metalloproteinase-3(MMP-3) or stromelycin-1 is a photolytic enzyme which play a pivotal role in joint. It is secreted by fibroblasts, synovial cells, and chondrocytes and is responsible for cartilage degradation. So we aimedto investigate the immunopathological and inflammatory or degenerative roles of Anti-MCV and MMP-3 in serum and synovial fluid (SF) of RA versusosteoarthritis (OA) patients.Patient and method: A total of 20 patients with RA, 20 patients with OA, and 10 control blood donors were included in the present study. CBC, ESR, CRP, RF, anti-MCV, and MMP3 were estimated in the blood of all subjects. In addition, synovial CRP, RF, Anti-MCV and MMP3 were estimated in both RA and OA patients.Results: The mean of ESR and both serum and SF CRP, RF, anti-MCV and MMP3 were significantly higher in RA group than OA group. In RA group, there was a significant positive correlation between serum anti-MCV and ESR, DAS28, serum of each of CRP, RF & MMP3 and synovial fluid anti-MCV, also, between serum MMP3 and serum anti-MCV and ESR. The sensitivity and specificity were found (75%, 80%), (95%, 90%) and (90%, 90%) for RF, anti-MCV and MMP3 respectively.Conclusion:Anti-MCV might be of a better diagnostic value for the early diagnosis of RA and its activity than RF, for its higher sensitivity and specificity detecting more aggressive and erosive disease. The elevated MMP-3 levels reflect disease activity and can be used as a specific degenerative marker for joints damage and deformity in both diseases, while more in RA than OA.

Keywords: RA, OA, Anti-MCV and MMP3

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, autoimmune, systemic, inflammatory disease mainly affecting the joints. It has a prevalence about 0.5% to 1% and an incidence of about 30 per 100 000 inhabitants, making it the most common chronic inflammatory autoimmune disease (14).RA is characterized by synovial proliferation and a symmetric erosive arthritis of peripheral joints with persistent symmetric polyarthritis (synovitis) that affect the hands, wrists and feet, although almost all diarthroidal joints may become involved. In addition to, articular manifestations, systemic involvement may cause constitutional symptoms; rheumatoid nodules, serositis and vasculitis. The severity of RA may fluctuate over time,

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but chronic RA most commonly results in the progressive development of various degrees of joint destruction, deformity, significant decline in functional status (36). Osteoarthritis (OA) is a common degenerative arthritis involving degradation of entire joint, including the subchondral bone, ligaments, capsule, synovial membrane and peri-articular muscles (9). OA is a highly prevalent and disabling disease that consequently has formidable individual and societal impact by impairing health related quality of life. Approximately 30% of the adult population has symptomatic OA (5).Rheumatoid factor (RF) is the most common diagnostic biomarker in RA. However, it has a fair sensitivity and a low specificity, since it is present in other rheumatic diseases, in infections, and in healthy people, especially the elderly (2).In seronegative cases of arthritis the differential diagnosis is not easily established in the early disease course. Therefore, the use of additional markers beside RF for early arthritis diagnosis is mandatory (56). The diagnosis of RA has been substantially improved by the introduction of standardized immunoassays for the detection of auto-antibodies against different citrullinated antigens (31).Anti-citrullinated protein antibodies (ACPAs) can be detected years before the onset of RA and reach a specificity of more than 95%. Therefore, in 2010, ACPA testing has become substantial part of the 2010 ACR-EULAR classification criteria for RA (1).Anti-mutated citrullinated vimentin(Anti-MCV) antibodies are member of ACPAs family, that result due to antibody production against antigens produced from the mutant citrullinated vimentin (43). Vimentin might trigger the initial immune response in synovial membranes by activating T-lymphocytes. Also, citrullinated vimentin increased in amounts in response to growth factors and pro-inflammatory cytokines, suggesting involvement in the pathophysiology of RA. That makes this protein an interesting auto antigen in RA (56). In addition, there are significant changes in radiograph scores in anti-MCV positive patients (52). The Matrix metalloproteinase-3(MMP-3) or stromelycin-1 is a proteolytic enzyme which is thought to play a pivotal role in joint. It secreted by fibroblasts, synovial cells, and chondrocytes. It is considered to be the most important proteinase responsible for cartilage degradation and resulting in deformity and disabling of joints (44).MMP-3 is locally produced in the inflamed joint, and released into the blood stream. Several studies have suggested that serum MMP-3 levels correlate with MMP-3 levels produced by the synovium, and thus reflect the level of activity of rheumatoid synovitis (number of clinically active inflamed joints)(26,37). MMP-3 may have diagnostic significance for the estimation of joint destruction. It would be valuable to have a marker that could be an indicator of joint damage progression, especially in the early stage of RA (44).

AIM OF THE WORK

This study aims to assess the immunopathological and inflammatory or degenerative roles of Anti-MCV and MMP-3 in RA by estimating their serum and SF levels in RA patients and comparing them to OA patients and normal controls.

SUBJECTS AND METHODS

A total of 50 subjects consisted of 20 patients with the diagnosis of RA, 20 patients with the diagnosis of OA and 10 control blood donors were included in this study.The study population was selected consecutively among patients who presented to the outpatient clinic and inpatient department of Rheumatology in Al-Hussein and Sayed Galal hospitals, cairo, Egypt, from Aug. 2011 till Apr.2013.All patients were subjected to complete history, full clinical examination with special attention to musculoskeletal system, and laboratory investigation and theywere chosen with a medical consent according to the ethical committee of Al-azhar University. The criteria used to determine cases include Patients that had not subjected to knee tapping or aspiration for at least six months before recruitment and

had not received either hyalouronan intra-articular knee injection or corticosteroid or immunosuppressive treatment for at least 3 months prior to the study and all RA & OA patients must be with effusion of at least one knee joint. The exclusion criteria included age below 16 years (Juvenile populations), other musculoskeletal conditions or disabilities or other autoimmune diseases and Patients with known comorbidity of chronic infection, blood diseases, metabolic diseases or malignancy.

The demographic data of the studied groups are shown in table (1).

		Control N=10	OA N=20	RA N=20
Gender	**(female/mal)**	6/4	13/7	14/6
	Female %	60%	65%	70%
Age(Years)	**(M±SE)**	40.5±2.97	55.4±1.68	47.3±2.9
BMI(Kg/m2)	**(M±SE)**	26.97±1.18	29.9±0.56	27±0.92

RA group.RA patients were diagnosed according to the ACR revised criteria (6). The diagnosis was confirmed by ACR/EULAR 2010 criteria (1).RA patients received prior medications; at the sampling time included 5mg of prednisolone, Methotrexate (7.5 mg/week), 200 mg hydoxychloroquine and non-steroidal anti-inflammatory drugs (NSAIDs). Patient assessment of pain was measured by visual analogue scale (VAS), ranging from 6-9. Patient assessment of function was measured by health assessment questionnaire (HAQ), ranging from 1.1-2.8 and disease activity was measured by disease activity score 28 (DAS28), ranging from 3.7-7.8.

Osteoarthritis group. OA patients were diagnosed according to criteria for diagnosis of OA by the ACR (4).OA patients received prior medications mostly NSAIDs. Patient's assessment for pain was measured by VAS and patient assessment of function (disability assessment) was measured by the Western Ontario and McMaster Universities index (WOMAC index).

Specimen collection and Laboratory Investigations: All subjects had their preferable blood samples for CBC and ESR. The sera and synovial fluids of patients were taken for hsCRP, RF, Anti-MCV and MMP3 by ELISA kit (eBioscience). Statistical analysis. Graph Pad Prism program version 5.0 was used for analysis of data. Data were summarized asmean ± SE. Mann- Whitney test and Kruskal-Wallis test were used for analysis of more than two variables which are not normally distributed,followed by Student's t-test.The ROC (receiver operating characteristic) curve is used to evaluate the performance of classification schemes in which there is one variable of two categories by which subjects are classified. Simple linearcorrelation (Pearson's correlation) was alsocarried out. P-value of up to 0.05 was considered significant.

RESULTS

As shown in table (2 and 3), the mean serum of each of ESR, CRP, RF Anti-MCV and MMP3 were significantly higher in RA group than both OA and control groups (p<0.0001, 0.0007, 0.006, p<0.0001 and <0.0001) respectively and the mean synovial fluid of each of CRP, RF, anti-MCV and MMP3 were significantly higher in RA group than OA group (p 0.002, <0.0001, 0.028 and 0.01) respectively.

Groups variables	Control (n=10)	OA (n=20)	RA (n=20)	P-value
Serum ESR(mm/hr)	10.6±1.90	23.15±4.43	80±10.31	<0.0001***
Serum CRP (mg/L)	4.01±0.64	5.26±0.89	13.96±2.26	0.0001
Serum RF (IU/ml)	3.53±0.55	5.43±0.55	87.28±19.19	0.006
Serum Anti-MCV (U/ml)	27.99±2.11	14.04±3.79	667.7±116.4	<0.0001**
Serum MMP3 (ng/ml)	2.02±0.49	4.85±1.24	10.04±1.12	<0.0001**

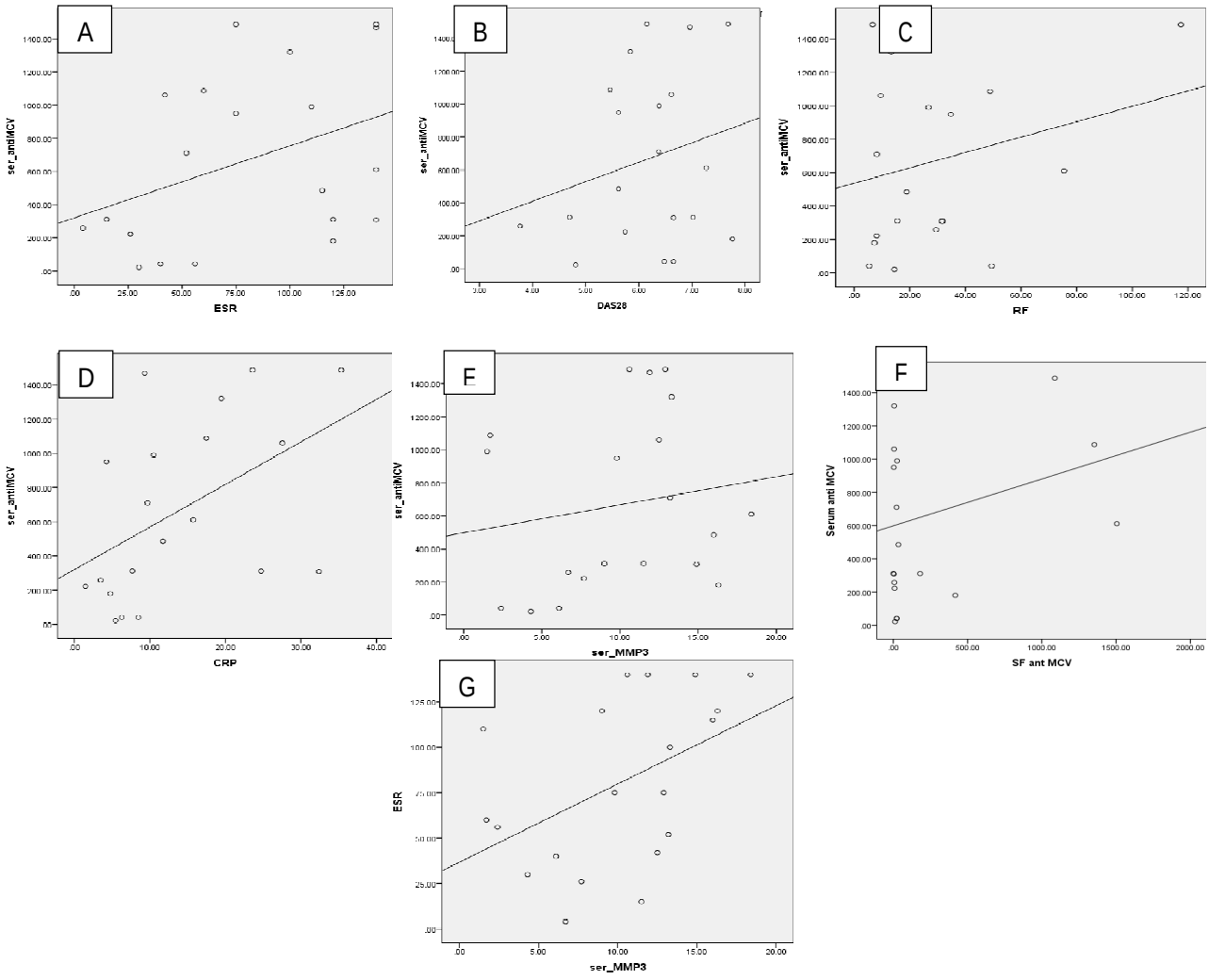
Table(2): Serum of each of ESR, CRP, RF,Anti-MCV & MMP3 in RA, OA and control group.

Data are represented as means + SE. *Indicate statistical significance.; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; RF,

Rheumatoid factor; Anti-MCV, Anti-mutated citrullinated vimentin; MMP3, Matrix metalloproteinase.

Groups variables	OA (n=10)	RA (n=20)	P-value
Sf. CRP (mg/L)	6.85±1.51	20.69±4.02	0.002
Sf. RF (IU/ml)	5.83±1.12	35.87±9.17	<0.0001**
Sf. Anti-MCV (U/ml)	19.54±8.33	244.78±106.6	0.028
Sf. MMP3 (ng/ml)	9.75±1.59	15.11±1.19	0.0106

Table (3): Synovial fluids of each of CRP, RF, Anti-MCV & MMP3 in both RA and OA control groups



Data are represented as means + SE. *Indicate statistical significance.; Sf, synovial fluids; CRP, C-reactive protein; RF, Rheumatoid factor; Anti-MCV, Anti-mutated citrullinated vimentin; MMP3, Matrix metalloproteinase.

In RA group, there was a significant positive correlation between serumanti-MCV and serum of each of ESR, DAS28, CRP, RF, MMP3 and synovial fluid anti-MCV (p=0.017, 0.050,0.003, 0.0003, 0.043 and 0.029 respectively) and between serum MMP3 and ESR (p=0.038) as shown in figure (1).

Figure(1): Correlation between serum Anti-MCV and serum of each of ESR (A), DAS28 (B), CRP (C), RF (D), MMP3(E) and synovial fluid anti-MCV (F)&between serum MMP3 and ESR (G).

ROC analysis of serum RF, anti-MCV & MMP3 were performed between RA patients & control to determine its diagnostic accuracy. It was found that, Area under the ROC curve was

0.7500, 09950 and 0.9475 respectively. The best cutoff for RF was 5.295 IU/ML which yielded sensitivity, specificity and LR of 75%, 80% and 3.75 respectively (p <0.0278).The best cutoff for anti-MCV was 22.20 U/ml which yielded sensitivity, specificity and LR of 95%, 90% and 9.5 respectively (p<0.0001). The best cutoff for MMP3 was 4.1 ng/ml which yielded sensitivity, specificity and LR of 90%, 90% and 9.0 respectively (p<0.0001) table (4) figure(2)

	RA & Control		
	Serum RF	**Serum Anti-MCV**	**Serum MMP3**
Cut off	5.295	22.20	4.6
AUC	0.7500	0.9950	0.9250
Upper limit	0.9398	1.011	1.016
Lower limit	0.5602	0.978	0.8338
Sensitivity	75%	95%	80%
Specificity	80%	90%	90%
LR	3.75	9.5	8.0
***P*-value**	0.0278*	<0.0001***	0.00018***

Table (4): ROC curves of Different studied parameters in RA & control groups:

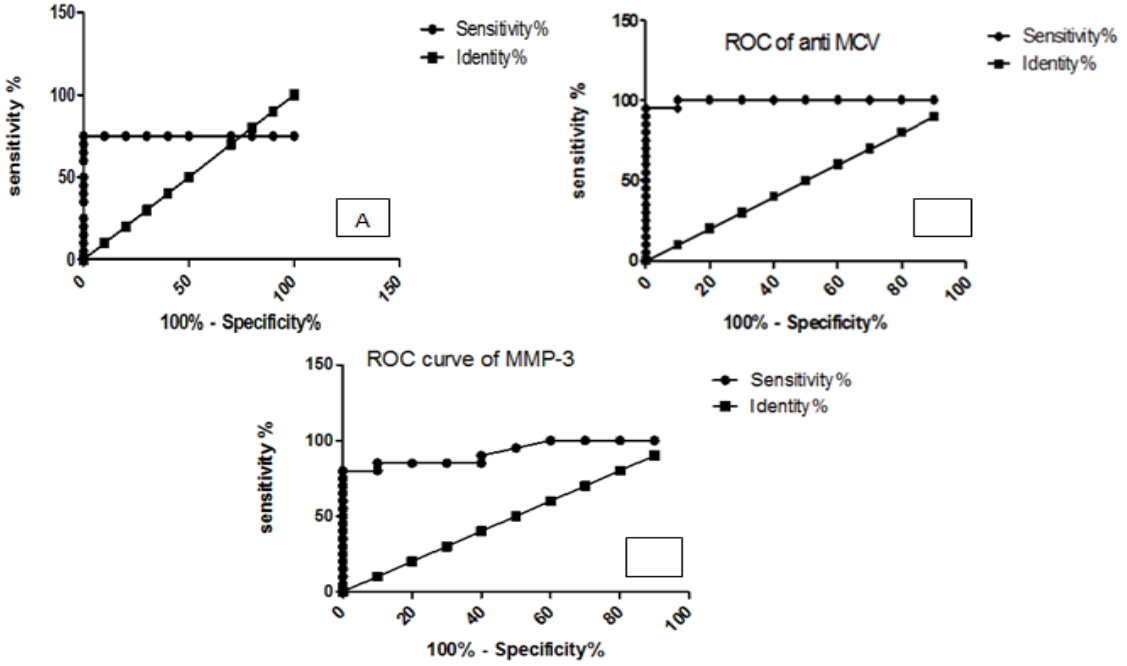


Figure (2): ROC curves of RF (A), Anti-MCV (B) and MMP3 (C) in RA group.

DISCUSSION

Rheumatoid arthritis is the most common chronic, progressive inflammatory disorder affecting synovial joints and leading to inflammation-induced comorbidities (46). The severity of RA may fluctuate over time, but chronic RA most commonly results in the progressive development of various degrees of joint destruction, deformity, significant decline in functional status and a premature death (39).

Osteoarthritis is a chronic degenerative disorder characterized by cartilage loss. Its prevalence is high. The cause of OA is not known; however, current evidence indicates that it is multifactorial with inflammatory, metabolic and mechanical causes. OA causes pain, stiffness, fatigue and functional impairment resulting in the disability, which seriously affects their quality of life and brings about heavy burden to the society and family (35).

RF is a very old serological marker for diagnosis of RA. RF is taken as a non-specific marker of RA because it is also seen in other

collagen vascular diseases like SLE and Sjögren's syndrome as well as in normal healthy individuals (47).

Anti-citrullinated protein antibodies have been reported as more specific serological markers of RA. They provide a superior alternative to the RF test in laboratory diagnostics of RA (21). Anti-MCV is an anti-citrullinated antibody reacting with mutated citrullinated vimentin. Citrullinated vimentin, identified as a new member of the ACPA family. Vimentin is secreted and citrullinated by macrophages depending on the proinflammatory signals, so anti-MCV antibodies have been considered highly specific diagnostic markers for RA. Additionally, the appearance of anti-MCV antibodies often precedes the development of RA (37).

As important as the anti-MCV antibodies in the radiological changes and even before the appearance of articular symptoms, the anti-MCV antidodies play an important role in the pathogensis of RA (8).So, our research tried to focus on the ability of anti-MCV to be considered as early diagnostic marker in Egyptian patients with RA.In our study, the mean level of RF as a laboratory tool, was a significantly higher in serum of RA patients than both OA patients and control group (P =0.0008) and in the synovial fluid of RA patients than OA patients.These results were in accordance with Capsi et al., (11), Hui et al., (21) and Khalifa and Abdelfattah, (25), but our results showed higher results than Kudo-Tanaka et al., (27).

In the current study, the mean serum level of anti-MCV showed highly significant difference in the RA than both OA group and control group (P<0.001) and synovial fluid of anti-MCV was significantly higher in RA than OA group (P<0.01).These results were supported by Al-Shukaili et al., (3), Bang et al., (8),Lui et al., (29),Snir et al., (48).

The work of Baeten et al.,(7) described that Intracellular citrullinated proteins are specific for RA synovial tissue. Moreover, De Rycke et al., (13) found that the presence of RA-specific synovial intracellular citrullinated proteins was associated with significantly higher systemic and local ACPA titers. These findings indicate that citrullinated vimentin is the predominant citrullinated antigen in SF of RA patients. This leads to the hypothesis that either: (i) citrullinated vimentin shows a higher affinity with ACPA; or (ii) that intracellulars with citrullinated vimentin show an insufficient clearance, which could result in a sustained inflammation (34) this may explained our higher results of anti-MCV in both serum and synovial fluid of RA patients.

The presence of citrullinated vimentin in the joint, its intracellular localization and the necessity of citrullination of vimentin clearly show the importance of citrullinated vimentin in the pathogenesis of inflammation in RA.

In the present study, serum Anti-MCV showed significant positive correlation with ESR(r=0.5255,p=0.0173), DAS28(r=0.445,p=0.050), CRP(r=0.6206, p=0.0034), RF(r=0.722, p=0.0003) and sf. anti-MCV(r=0.4857,p=0.0299) in RA group.These results were parallel to that ofDejaco et al.,(12), Innala et al., (22), Mathsson et al., (34) and Ursum et al., (55) who reported a significant correlation between anti-MCV antibody titers and both the severity of RA and the disease-activity score(DAS28).Also,Bizzaro, (10) and Keskin et al., (24) had been documented that anti-MCV anti-bodies were correlated with disease activity parameters such as DAS28, ESR, CRP levels and serum RF levels.

The work of Mathsson et al.,(34) and Wagner et al., (56) showed a strong correlation of levels of anti-MCV with clinical parameters (ESR, swollen joint count, physician's assessment of disease activity and DAS28), making anti-MCV a better prognostic marker for future radiographic changes.

In this study, the sensitivity and specificity of RF were 75% and 80%, respectively (P < 0.01) and the sensitivity and specificity of anti-MCV were 95% and 90%, respectively (P < 0.0001).Our results were higher than, as regard RF sensitivity in Al-Shukaili et al., (3) (57%) and Liu et al.,(29) (72.4%), while lower than Poulsom and Charles,(42) regarding the

sensitivity (84%) andAl-Shukaili et al.,(3) andPoulsom and Charles, (42) regarding the specificity (94% and 87%) respectively and parallel with Liu et al.,(29) regard specificity (80.1%).

Also, our results were higher as regard anti-MCV sensitivity inAl-Shukaili et al.,(3),Luime et al., (30) and Renger et al., (43) (72%), Liu et al.,(29) (87.2%) and Lopez-Longo et al.,(28) (82%) and higher regard specificity (87%) inAl-Shukaili et al., (3) and (88%) Bang et al.,(8) and Poulsom and Charles,(42), while lower than Liu et al., (29),Luime et al., (30) and Renger et al., (43) regard specificity (93.4%, 99.7% and 99%)respectively.

We observed that in agreement with our study, most studies done on that approach concluded that anti-MCV antibodies had higher sensitivity and specificity than RF in the diagnosis of RA. The difference in sensitivity and specificity in all these studies may be due to the different cut off point used and the different number of patients contained in each study.

MMP-3 (matrix-metalloproteinase- 3, stromelysin-1) is a member of the matrix metalloproteinases family and has a wide range of substrate specificities, e.g. tissue matrix proteins such as cartilage proteoglycans, fibronectin, various collagens and laminin(45). It has been shown to be expressed in OA and overexpressed in RA patients. It is also directly involved in cartilage and bone destructive processes, as it activates other degrading enzymes such as procollagenase (proMMP-1) and progelatinase B (proMMP-9) and is therefore thought to be a key player in joint destruction in OA and RA patients. In addition, it has been shown that MMP-3 is produced by articular synovial cells, fibroblasts and chondroblasts. It is secreted as an inactive zymogen (proMMP-3) which must be activated by endopeptidases. The serum and synovial fluid of RA patients contains large amounts of MMP- 3 (41).

It is known that MMP3 is expressed in OA synovium; however, there is little evidence available on the difference of MMP3 expression in synovial membrane at different stages of OA, Producing “waterfall-like” amplification effect (19, 20).

In the present study we found that MMP-3 expression in serum and synovial of OA patient was increased compared with control group. Our study was supported by Fernandes et al.,(15), Okada et al., (38),Tetlow et al., (54) and Yoshihara et al., (58)that showing high level of MMP3 in OA patients compared with control group. This evidence indicated that MMP3 is progressively increasing in OA and highlighted the contribution of MMP3 in OA pathogenesis (15).Also, this evidence suggests that MMP-3 play a potential crucial role in OA pathogenesis, and MMP-3 inhibitors can delay OA or remove synovial lesions by arthroscopic surgery. This can not only relieve pain and improve function, but also reduce inflammatory factors production and slow down OA, so that patients will avoid premature artificial joint replacement and the resulting high cost. In addition, MMP-3 is considered an indicator of early diagnosis and disease activity of OA patients (23).So, we found that MMP3 was expressed in OA serum and synovium, suggesting that MMP3 expression is closely linked with the degradation of joint tissue in OA patients.

In the present study, the mean activity of MMP3 was significantly higher in RA group than OA and control groups ($p<0.0001$).These results were in accordance with Kobayashi et al.,(26), Marcel et al., (33), Poole et al., (41),Tchetverikov et al., (53), Yamanaka et al., (57) and Yoshiharaa et al., (58).

In this study, the mean synovial fluid level of MMP3 was significantly higher in RA group than the OA group ($p=0.0106$).These results were in accordance with Poole et al.,(41),Syversen et al., (52),Yoshiharaa et al., (58) and Young-Min et al., (59) who found that synovial MMP3 show significant difference between RA patients and OA patients, also, the study of Kobayashi et al.,(26) and Ribbens et al., (45) indicated significantly higher synovial fluid concentration of MMP3 in combination with high serum levels in RA patients than OA patients.

Our results explained that MMP3 a degenerative marker, is expressed in OA as a degenerative disease also, the MMP3 is highly expressed in RA which may reveals the degenerative features of this sever inflammatory disease. Depending upon our results, the anti-MMP3 may be used as a therapeutic agent that may decrease the joint degradation in OA as well as using the MMP3 as a follow up marker for RA progression, that may be need further studies.

In the present study, there was a significant positive correlation between MMP3 and ESR in RA group ($r = 0.467$, $p = 0.0377$).In support to our results Green et al.,(16) and So et al., (49) found a strong positive correlation between serum MMP3 levels and ESR in RA patients. Confirming our results Peak et al.,(40) who measured levels of MMP3 in paired synovial fluid and serum and correlated their levels to the standard measures of ESR ($r=0.41$, $p=0.01$). In this study, the sensitivity and specificity of MMP3 were 80% and 90%, respectively and this was higher than Hayashi and Kumagai,(17)regarding sensitivity (66.7%), also lower than Hayashi et al.,(18) regarding specificity (92%).(50) reported that MMP3 sensitivity and specificity were lower than our study (75.7% and 49.5 %) respectively. The difference in sensitivity and specificity in all these studies may be the different cut off point used and the different number of patients contained in each study.

CONCLUSION:

Anti-MCV might be of a better diagnostic value for the diagnosis of RA than RF for its high sensitivity and specificity thus anti-MCV can be a promising and reliable predictor of radiological changes, activity and early diagnosis of RA and it detecting more aggressive and erosive disease. The elevated MMP-3 levels reflects disease activity and can be used as a specific degenerative marker for joints damage in both OA and RA in different degrees reflecting more degeneration and joints deformity in RA than OA.

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