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Anti-mutated citrullinated vimentin antibodies in rheumatoid arthritis; diagnostic utility and association with deformities and disease activity

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Abstract

Rheumatoid arthritis (RA) is a chronic autoimmune disease with multiple morbidity burdens. Early diagnosis of RA is the main key in management and prevention of disease complications. Much research nowadays is looking for a serological marker with high accuracy in diagnosis of early RA cases. Our aim in this study was to evaluate the role of anti-mutated citrullinated vimentin (anti-MCV) antibodies in the early diagnosis of RA. In addition to compare its diagnostic sensitivity and specificity with anti-cyclic citrullinated peptide antibodies (anti-CCP) and RF antibodies in early versus established RA patients. This prospective cross-sectional study included 80 participants: 40 RA patients (20 early RA patients and 20 established RA patients), 20 patients with other rheumatic diseases (as a disease control group), and 20 apparently healthy participants as normal controls. All participants underwent history taking, clinical examination (general, articular assessment and calculation of disease activity score (DAS28-ESR)) for RA patients, radiological and laboratory investigations (RF, anti-CCP2 and anti-MCV antibodies measurements by ELISA technique). The results showed that the mean values of anti-CCP2 and anti-MCV were significantly increased in RA cases compared to the control groups (p=0.00 and p=0.01, respectively). Anti-MCV had sensitivity and specificity of 63% and 83%, respectively for diagnosing of early RA at area under curve of 0.80 compared to sensitivity and specificity 37% and 100%, respectively for anti-CCP2. Also, both (anti-CCP2 and anti-MCV) had positive significant correlations with ESR (p<0.001 and p=0.02, respectively), CRP (p=0.01 and p=0.02, respectively) and DAS 28 (p<0.001 for both). In conclusion, our data indicated that anti-MCV antibodies may represent a valuable marker for diagnosis of early RA cases.

Keywords: RA, RF, Anti CCP, Anti MCV, DAS-28.

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Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease, with progressive destructive polyarthritis and systematic manifestations. It is associated with significantly high morbidity and mortality rates with functional capacity impairment.[1, 2] Not only it impairs patients' quality of life,[3] but also patients with RA and other inflammatory joint

disorders (IJD) have increased cardiovascular disease (CVD) risk compared with the general population.[4] It is characterized by proliferative synovitis, which involves angiogenesis, infiltration of lympho-monocytes and production of pro inflammatory cytokines which lead to chronic destruction of the joints.[2]. Autoantibodies can be silent for up to 15 years. Thus, patients may have systemic autoimmunity but show no symptoms, some patients may then progress to an aggressive form of the disease, while others may have a mild form of RA. [5]

Numerous serological markers of RA have been described, among these; anti-citrullinated protein autoantibodies (ACPA) that are more specific serological markers than RF in diagnosis of RA. Other markers include anti-cyclic citrullinated peptide (anti-CCP) antibodies and anti-mutated citrullinated vimentin (anti-MCV) antibodies.[6] One of the most common assays in clinical practice is the determination of second-generation anti-citrullinated cyclic peptide antibodies (anti-CCP2)[7] that was included in the new RA classification criteria.[8] Moreover, the combination of anti-CCP and RF positivity would increase the probability of an accurate positive result compared to performing a single test.[9, 10] Nevertheless, anti-CCP sensitivity is limited to around 38% in patients with RA disease.[11, 12]

Anti-MCV is the newest member of this autoantibodies family,[13] it is proposed as a tool for RA diagnosis.[14, 15] To date, there is controversy whether anti-MCV assay can be used as a tool for diagnosis and prognosis of RA disease compared to anti-CCP antibodies.[7, 13-16]

Furthermore, it is not clear if anti-MCV antibodies are correlated with disease activity score or other parameters of activity e.g. ESR as different studies were conducted with various results.[6, 17] Therefore, the aim of this study was to evaluate the role of anti-MCV antibodies in early diagnosis of RA. In addition to compare its diagnostic sensitivity and specificity with anti-CCP and RF antibodies in early versus established RA patients.

Subjects and Methods

This cross-sectional study included 80 participants, recruited from Assiut University Hospital, Internal Medicine Department, and clinics. Of these, 40 patients with RA were diagnosed according to 2010 American College of Rheumatology (ACR) classification criteria for RA[8] (20 early RA patients (<1 year) and 20 established RA patients (>1year)). And 20 patients were selected to represent other rheumatic diseases (11 patients with systemic lupus erythematosus, 6 patients with osteoarthritis and 3 patients with psoriasis as a disease control group). Finally, 20 apparently healthy participants were recruited as the normal control group.

Patients with Crohn's disease, ulcerative colitis, malnutrition, hyperparathyroidism, hyperthyroidism, renal, hepatic diseases, any limitation of physical activity or medications that might affect bone metabolism or the endocrine system (e.g., thyroxin, anticonvulsants, hormone, or vitamin D replacement therapy), malignancy, chronic infection as TB, Hepatitis B and C, and mixed connective tissue diseases were excluded from our study.

Ethical considerations

This study protocol was reviewed and approved by the ethics Institutional Review Board committee of the Faculty of Medicine, Assiut university (dated September 2016). The clinical trial identifier number of the current study: NCT0322437. Before being included in the study, all study subjects gave their informed consent to participate in the study.

Assessment of disease activity score

All patients underwent history taking, clinical examination (general and articular assessment). Disease activity status was assessed for RA patients using the disease activity score-28 (DAS-28-ESR). The components of DAS-28-ESR were swollen and tender joints counts (each 0–28), patient-assessed global score (0–100), and erythrocyte sedimentation rate (ESR). Using disease activity score calculator (DAS28) according to Van Gestel et al., 1998, DAS28-*ESR (three variables*) = (0.56×√ [TJC28] + 0.28×√ [SJC28] + 0.70× ln [ESR]) ×1.08 + 0.16.[18] High disease activity was defined as a DAS28-ESR > 5.1, moderate disease activity was defined as

3.2 < DAS28-ESR ≤ 5.1, low disease activity was defined as 2.6 ≤ DAS28- ESR ≤ 3.2 and remission as a DAS28-ESR < 2.60.[18,19] Patients with deformities were clinically examined by expert rheumatologist to identify what type of deformities was presented.

Laboratory Tests

The following investigations were done to all study participants, including complete blood count (CBC) using an automated blood counter (Ruby CELL DYN, Abbott, Germany), ESR using an analyzer (Alifax® analyzer, Sysmex, USA), urine analysis, kidney and liver function tests using an automated chemistry analyzer (Dimension RXL MAX, Germany), C-reactive protein (CRP) using an automated analyzer (ADVIA 1800 SIEMENS Healthcare Diagnostics Inc, USA). RF was measured in sera by latex enhanced immunoturbidimetric method (ADVIA 1800, SIEMENS Healthcare Diagnostics Inc., UK).

Anti-CCP was measured using enzyme-linked immune-sorbent assay (ELISA) commercial kits for qualitative and semi quantitative detection of IgG anti-CCP antibodies in human sera (Architect i1000SR (Abbott, Germany). The normal reference level was up to 0.5 U/ml. Anti-MCV was measured using ELISA commercial kits for the quantitative measurement of IgG class anti-MCV autoantibodies in human serum using a commercial ELISA Kit (Cat. No: CSB-E09565h; Cusabio, Hubei, China), according to the manufacturer's instructions.

Radiological Tests

Standard X-ray was performed for small joints of the hands, wrists, feet, and the other affected joints using a machine (COLLIWATOR R-20J, SHIMADZU CORPORATION, Japan).

Statistical analysis

Data was collected and analyzed using SPSS (Statistical Package for the Social Science, version 20, IBM, and Armonk, New York). Continuous data was expressed in form of mean ± SD or median (range) while nominal data was expressed in form of frequency (percentage). Chi square-test was used to compare the nominal data of different groups in the study while student t-test was used to compare mean of different two groups and ANOVA test for more than two groups. Spearman correlation was used to determine the correlation between Anti-CCP2 and Anti-MCV with laboratory parameters. The receiver operating characteristic (ROC) curve was used to determine the diagnostic accuracy of anti-CCP2 and anti-MCV in diagnosing the RA. The significance level was set at $p< 0.05$.

Results

Demographic and clinical characteristics of the study groups

RA patients were 30 females (75%) and 10 males (25%), with mean age of 48.97 ± 9.08. The mean age of the disease control and normal control groups were 29.25 ± 6.18 and 29.05 ± 4.48 years, respectively. Other clinical data as sex, disease duration, swollen joints count, DAS-28-ESR and deformities are presented in Table 1.

Table 1. Demographic and clinical characteristics of RA Patients and Controls.

Item		RA group Mean ± SD or N (%)	Disease control* Mean ± SD or N (%)	Normal Control Mean ± SD or N (%)
Age		48.97 ± 9.08 (24- 65)	29.25 ± 6.18 20- 45	29.05 ± 4.48 (19- 63)
Sex	Male	10 (25%)	6 (30%)	6 (30%)
	Female	30 (75%)	14 (70%)	14 (70%)
Disease duration		3.45 ± 2.11	2.67 ± 1.21	-
Swollen joint count		12 (2- 22)	3 (1- 12)	-
DAS-28-ESR		5.09 ± 1.25 (2.5-7.45)	-	-

Table 1. Continued.

Item	RA group Mean ± SD or N (%)	Disease control* Mean ± SD or N (%)	Normal Control Mean ± SD or N (%)
Deformities			
No deformity	16 (40%)	20 (100%)	20 (100%)
Z deformity of the thumb	9 (22.5%)	0	0
Ulnar deviation of hand	6 (15%)	0	0
Boutonniere deformity	4 (10%)	0	0
Flexion deformity	3 (7.5%)	0	0
Swan neck deformity	2 (5%)	0	0

Data are expressed as mean (±SD), range, numbers, and percentages. *Disease control included patients with other rheumatic diseases

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Laboratory Data in the Studied Groups

The level of anti-MCV was significantly higher in the RA group versus disease control and normal control groups (p=0.01, p=0.03 and p=0.31, respectively). Also, anti-CCP2 and RF levels were significantly higher in RA group versus disease control and normal control groups (p=0.00, p=0.01 and p=0.34) and (p=0.01, p=0.00 and p=0.52, respectively). (Table 2)

Table 2. Laboratory Data of the Studied Groups.

Variables	RA group	Disease control*	Normal control	*p*1	*p*2	*p*3
CRP (mg/dl)	22.7 ± 10.78	29 ± 15.17	8.7 ± 3.72	NS	0.03	0.01
ESR (ml/h)	55.67 ± 22.12	49.09 ± 21.78	13.23 ± 5.71	NS	0.04	0.02
RF (mg/dl)	23.70 ± 10.91	4 ± 1.32	0	0.01	0.00	NS
Anti-CCP2(u/ml)	59.16 ± 12.78	12.23 ± 3.42	10.09 ± 5.14	0.00	0.01	NS
Anti-MCV(u/ml)	53.15 ± 15.06	15.95 ± 4.02	13.41 ± 7.4	0.01	0.03	NS

Data are expressed as mean (±SD), range. *p> 0.05 is not significant (NS). *Disease control included patients with other rheumatic disease. RA, rheumatoid arthritis, CRP; C- reactive protein; ESR, erythrocyte sedimentation rate; RF, rheumatoid factor; Anti-CCP; anti-cyclic citrullinated peptide; Anti-MCV, anti-mutated citrullinated vimentin; p1: comparison between RA group and disease control group; p2: comparison between RA group and healthy control group; p3, comparison between the disease control group and the normal control group.

Laboratory data of patients with early and established rheumatoid arthritis

Patients with established RA (>1 year) had significantly higher CRP, ERS, anti-CCP2 and anti-MCV in comparison to those with early RA (<1 year) (p=0.03, p=0.01, p=0.03 and p<0.001, respectively) (Table 3). There was no difference in RF results between the two groups (p= 0.98).

Table 3. Laboratory data in patients with early and established RA.

Variable	Early RA	Established RA	**p* value
CRP (mg/dl)	21.40 ± 10.27	24.15 ± 11.27	0.03
ESR (ml/h)	51.07 ± 20.62	59.11 ± 27.11	0.01
RF (mg/dl)	25.72 ± 8.09	21.92 ± 11.21	NS
Anti-CCP2 (u/ml)	51.07 ± 12.11	67.25 ± 10.12	0.03
Anti-MCV (u/ml)	31.15 ± 9.16	75.15 ± 9.13	0.001

Data are expressed as mean (±SD), range. ***p***> 0.05 is not significant (NS)**.**

Diagnostic accuracy of RF, anti-CCP and anti-MCV and in diagnosis of patients with RA disease (Early vs. Established RA)

It showed that Anti-CCP2 had sensitivity 37% and specificity 100% for diagnosing early RA with area under curve 0.83 at a cutoff point > 10 u/ml, while anti-MCV had sensitivity 63% and specificity 83% for diagnosing early RA with area under curve 0.80 at a cutoff point > 17. RF had sensitivity 63% and specificity 53% for diagnosing of early RA with area under curve 0.65 at a cutoff point > 7 u/ml. As regard established RA; Anti-CCP2 had sensitivity 76% and specificity 100% with area under curve 0.96 at a cutoff point > 32 u/ml, while anti-MCV had sensitivity 67% and specificity 100% with area under curve 0.82 at a cutoff point > 31. RF had sensitivity 62% and specificity 65% with area under curve 0.67 at a cutoff point > 12 u/ml. (Table 4)

Table 4. Diagnostic accuracy of RF, anti-CCP and anti-MCV and in diagnosis of patients with RA disease (Early vs. Established RA).

Indices	Anti-CCP2		Anti-MCV		RF	
	Early	Established	Early	Established	Early	Established
Sensitivity	37%	76%	63%	67%	63%	62%
Specificity	100%	100%	83%	100%	53%	65%
PPV	100%	100%	63%	100%	39%	48%
NPV	77%	85%	83%	85%	75%	77%
AUC	0.83	0.96	0.80	0.82	0.65	0.67
Cutoff point (u/ml)	> 10	> 32	> 17	> 31	> 7	> 12
p value	< 0.001	< 0.001	<0.001	<0.001	< 0.001	<0.001

*$P \leq 0.05$ is significant.

Serum levels of anti-CCP, anti-MCV and RF in relation to clinical and laboratory parameters in RA patients

Table 5 shows that anti-CCP2, anti-MCV and RF were positively significantly correlated with DAS 28, ESR and CRP. It was noticed that anti-CCP2 had positive significant correlation with anti-MCV (r= 0.43; *p*< 0.001).

Table 5. Serum levels of anti-CCP, anti-MCV and RF in relation to clinical and laboratory parameters in RA patients.

Variables	Anti-CCP		Anti-MCV		RF	
	r	*p*	r	*p*	r	*p*
ESR (mm/hr)	0.55	<0.001	0.43	0.02	0.44	<0.001
CRP (mg/dl)	0.34	0.01	0.43	0.02	0.32	0.01
DAS 28-ESR	0.50	<0.001	0.45	<0.001	0.52	<0.001
Anti-CCP (u/ml)	------		0.43	0.001	0.50	<0.001
Anti-MCV (u/ml)	0.43	<0.001	-------		0.22	0.04
RF (mg/dl)	0.50	<0.001	0.22	0.04	----	
DAS 28 ESR	0.50	<0.001	0.45	<0.001	0.52	<0.001

Date are presented as r values, indicating the strength of correlation and p values, indicating the significance of correlation and considered significant if *p*<0.05. DAS, disease activity score; Anti-CCP; anti-cyclic citrullinated peptide; Anti-MCV, anti-mutated citrullinated vimentin; RF, rheumatoid factor.

Levels of anti-MCV according to deformities and disease activity in RA patients

Patients presented clinically with deformities had significantly higher levels of anti-MCV compared to those patients without deformity (p=0.03). Moreover, different disease activities of DAS-28-ESR showed significant differences in levels of anti-MCV (p=0.001) (Table 6).

Table 6. Differences in anti-MCV levels according to deformities and disease activity in RA patients.

Patients' characteristics	Patients' deformities		DAS -28-ESR			
	patients with deformities	patients without deformities	Remission	Low	Moderate	High
Level of Anti-MCV (U/ml)	57.56 ± 12.67	50.22 ± 10.45	11.40 ± 3.33	32.72 ± 10.01	53.91 ± 18.65	57.98 ± 12.1
*p value	0.03		0.001			

*$p \leq 0.05$ is significant.

Discussion

RA is a chronic, autoimmune, inflammatory systematic disease that affects synovial joints, leading to destruction and deformity. In general, it is not a fatal disease, but its complication such as cardiac and respiratory problems leads to increased mortality. Also due to the long-term disease complications, RA is associated with a reduced life expectancy.[20] So that the development of a sensitive and specific biomarker for diagnosis of RA, which could be detected in early disease, would enable RA patients to be identified, monitored, and treated appropriately.[14] Multiple autoantibodies had been discovered to be associated with RA as rheumatoid factor.[21]

RF is the first autoantibody correlated with RA. It is the commonly accepted and widely used serologic test for RA; however, it is not specific in diagnosing early RA because it may be present in normal elderly persons or in patients with other autoimmune and infectious diseases.[21] Anti-CCP assays are effective and widely used for diagnosing RA. However, their sensitivity is limited to 40% in patients with early RA[22] CCP is not expressed in the synovium and citrullinated proteins expressed in the rheumatoid joint would probably be more relevant as targets of autoantibodies used to diagnose RA. Vimentin is an intermediate filament that is widely expressed by mesenchymal cells and macrophages and easy to detect in the synovium.[23]

In the current study, there was a significantly higher level of serum anti-MCV antibody in RA patients compared to healthy and disease control groups, it was supported by previous studies,[14, 24,25] this could be explained by the hypothesis that vimentin might trigger the initial immune response in RA. It activates T lymphocytes by binding on HLA-DR4 on the surface of antigen presenting cells and may contribute to certain pathways in the pathogenesis of RA.[26]

Our findings in the present study confirmed observations of previous studies that anti-CCP assays had superior specificity than anti-MCV antibodies in early RA.[6, 27] Nevertheless, in terms of a reliable screening test for RA disease, a higher sensitivity is extremely required to establish the earlier diagnosis. Our data agree with the observation of a previous study that anti-MCV had higher sensitivity than anti-CCP assays in early RA.[10] This finding could be explained by a hypothesis that vimentin contains 43 arginine residues, each arginine residue can potentially be citrullinated by peptidylarginine deiminase (PAD) resulting in a variety of citrullinated epitopes. On the other hand, anti-CCP2 has only a few epitopes.[7, 33]

In contrast to our findings, regarding patients with early RA disease, anti-MCV did not sustain its diagnostic performance and anti-CCP

showed superior sensitivity compared to anti-MCV.[28] This could be attributed to that ACPA specificities broaden with time.[29] Also, it is well known that antibodies levels can be changed variably following disease-modifying antirheumatic drugs (DMARDS)[30] and anti-MCV antibodies levels decreased more rapidly than anti-CCP levels.[31] A study by Innala et al., 2008, observed that anti-MCV antibodies assays in early RA declined in response to treatment.[17] Interestingly, it was noticed the heterogeneity of the decline of different ACPA assays following anti-rheumatic medications and the anti-MCV antibodies were the most affected autoantibodies.[32] As for RF assay in early RA, our findings demonstrated the inferior specificity of RF than both anti-CCP2 and anti-MCV antibodies measurements. On the other hand, RF demonstrated similar and superior sensitivity compared to anti-MCV and anti-CCP, respectively.

Our study indicated that in established RA, RF levels showed inferior specificity and sensitivity than either anti-CCP or anti-MCV antibodies measurements. To date, few studies were conducted in early RA of less than 1 year, and showed that anti-CCP2 demonstarted either similar or higher specificity compared with anti-MCV for RA but higher or lower sensitivity compared with anti-MCV for RA.[13, 17, 27] When compared to RF, anti-MCV had demonstrated either higher or lower sensitivity and higher, equal, or lower specificity for RA.[13, 17, 27]

It is worth mentioning that a study performed by Barouta et al., 2017, which investigated the diagnostic value of anti-MCV in patients with very early RA disease (<3 months duration), anti-MCV showed higher specificity and sensitivity than anti-CCP2 but equal specificity with RF.[28] Previous studies showed variable results in established patients with RA disease, some of which showed that anti-MCV specificity either inferior[6, 16, 34-35] or equal compared to anti-CCP specificity.[28, 36, 37, 38.] Interestingly, Díaz-Toscano et al., 2017,[7] found that anti-MCV specificity was higher than anti-CCP when patients with other rheumatic diseases served as controls. For sensitivity, previous studies reported different findings, some studies found that anti-MCV antibodies sensitivity were inferior,[28, 34, 37] others superior[7, 38, 41, 42] or equal[16, 36, 39] to anti-CCP sensitivity. Furthermore, studies also reported variable results in both specificity and sensitivity of RF compared to anti-MCV antibodies.[16, 31, 34-36, 43]

Obviously, the diagnostic value of anti-MCV test by itself and compared to anti-CCP and RF assays demonstrated variable different results. These discrepancies may be attributed to various factors including different patient ethnics origin, methodology, different kits used[44] different cutoff values,[16,18, 34, 37] heterogeneity in selecting the control groups,[42, 45] different assays of anti-CCP antibodies measurements[29, 46, 41] or using different cutoff points of anti-MCV antibodies which could give different results.[16]

For example, Barouta et al., 2017, found that anti-MCV antibodies measurements demonstrated the highest sensitivity and specificity when the cutoff value for anti-MCV in their study was 42 U/ml. But when the cutoff value was set at 20 U/ml, they found higher anti-MCV sensitivity but lower specificity compared to anti-CCP.[28.]

Furthermore, in our study, we tested only the anti-CCP2 assay, but we did not include anti-CCP3 antibodies measurements. Anti-CCP3 assays depend on additional epitopes that are not present in the anti-CCP2 antigen sequence.[29, 46] Therefore, the measurement of anti-CCP3 antibodies may results in an increase in sensitivity compared to that observed in anti-CCP2 in our study.[7] However, other systemic reviews showed no difference in utility values when comparing anti-CCP2and anti-CCP3 assays.[47, 48]

Our data indicated that anti-MCV antibody levels were associated with RA disease activity parameters like ESR, CRP, and DAS-28-ESR; prognostic for disease severity[17-24, 49] and levels of serum RF.[6, 13, 24] These findings could be attributed to their potential role in the pathogenesis of RA. However, different studies resulted with variable outcomes.[6, 32, 38, 40] Such differences may be associated with differences in study design, study methodology or due to differences in patient clinical disease activity.

The considerable association of anti-MCV antibodies with other autoantibodies would imply their consistent diagnostic and prognostic role. In the present study, our findings confirmed a significant positive association between anti-MCV and anti-CCP2 in patients with RA. This agreed with previous studies.[6, 13, 30, 43] This finding can be explained by that cross-reactivity experiments between anti-MCV and anti-CCP2 antibodies indicated that both target some shared epitopes which may explain the high positive correlation between these antibodies.[50]

As a novelty, our results showed a positive association between anti-MCV measurements and patients with RA disease presented with deformities. This finding points to the important role of anti-MCV positivity in disease severity and joint damage. To date, no previous study investigated the association between anti-MCV levels and patients with RA disease, presented with clinical deformities. This can be explained that high levels of anti-MCV were related to higher serum levels of bone and cartilage destruction markers and anti-MCV induce osteoclastogenesis and bone loss [51]. Consequently, we hypothesize that early detection of high anti-MCV in patients with RA disease may predict severe deforming disease and necessitate earlier aggressive treatment.

Our study had certain limitations. These included the small number studied patients and the study design, cross-sectional. Longitudinal studies would result in examining changes over time. Furthermore, we did not study associations with extra articular manifestations, and did not include an anti-CCP-3 assay to rely upon additional epitopes, not present in the anti-CCP2 antigen sequence.

In conclusion, anti-CCP2 antibodies had higher diagnostic specificity in both early and in established RA disease. However, anti-MCV displayed superior sensitivity in screening early RA compared to anti-CCP2. Testing of anti-MCV antibodies for screening of RA disease especially in the early stage of the disease can guide the choice of initial therapy, reserving aggressive regimens for those with high anti-MCV levels and who are suspected to have an aggressive course and deformities.

Author Contributions

NMT, HSHED, GHA, SKS and ASA; conceptualization, data curation, formal analysis, methodology, visualization, writing original draft, writing review and editing. SKS, conceptualization, methodology and performed the laboratory investigation. NMT, HSED, SKS supervision; read and agreed to the published version of the manuscript. The authors read and approved the final manuscript**.**

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

This study protocol was reviewed and approved by the ethics Institutional Review Board committee of the Faculty of Medicine, Assiut university (dated September 2016). The clinical trial identifier number of the current study: NCT0322437.

Informed consent

Before being included in the study, all study subjects gave their informed consent to participate in the study.

References

1. Andrews, NP., Fujii, H., Goronzy, JJ., et al., (2010). Telomeres and immunological diseases of aging. *Gerontology*, 56:390-403.

2. Gamal, RM., Hammam, N., Zakary, MM., et al., (2018). Telomere dysfunction-related serological markers and oxidative stress markers in rheumatoid arthritis patients: correlation with diseases activity. *Clin Rheumatol*, 37:3239-46.

3. Gamal, RM., Mahran. SA, El Fetoh, NA., et al., (2016). Quality of life assessment in Egyptian rheumatoid arthritis patients: Relation to clinical features and disease activity. *The Egyptian Rheumatologist*, 38:65-70.

4. Agca, R., Heslinga SC., Rollefstad, S., et al., (2017). EULAR recommendations for cardiovascular disease risk management in patients with rheumatoid arthritis and other forms of inflammatory joint disorders. update. *Ann Rheum Dis*, 76: 17-28.

5. Smolen, JS., Breedveld, FC., Burmester, GR., et al., (2016). Treating rheumatoid arthritis to target: 2014 update of the recommendations of an international task force. *Ann Rheum Dis*,75: 3-15.

6. El Shazly,RI., Hussein, SA., Raslan, HZ., et al., (2014). Anti-mutated citrullinated vimentin antibodies in rheumatoid arthritis patients: Relation to disease activity and manifestations. *The Egyptian Rheumatologist*, 36; 65-70.

7. Díaz-Toscano, ML., Olivas-Flores, EM., Zavaleta-Muñiz, SA., et al., (2014) Comparison of two assays to determine anti-citrullinated peptide antibodies in rheumatoid arthritis in relation to other chronic inflammatory rheumatic diseases: assaying anti-modified citrullinated vimentin antibodies adds value to second-generation anti-citrullinated cyclic peptides testing. *BioMed research international*, 2014:198198.

8. Aletaha, D., Neogi, T., Silman, AJ., et al., (2010). Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Ann Rheum Dis*, 69: 1580-8.

9. Reitsma, JB., Glas, AS., Rutjes, AW., et al., (2005). Bivariate analysis of sensitivity and specificity produces informative summary measures in diagnostic reviews. *J Clin Epidemiol*, 58; 982-90.

10. Mathsson Alm, L., Fountain, DL., Cadwell, AM et al., (2018). The performance of anti-cyclic citrullinated peptide assays in diagnosing rheumatoid arthritis: a systematic review and meta-analysis. *Clin Exp Rheumatol*, 36; 144-52.

11. Taylor, P., Gartemann, J., Hsieh, J., et al., (2011). A systematic review of serum biomarkers anti-cyclic citrullinated Peptide and rheumatoid factor as tests for rheumatoid arthritis. *Autoimmune Dis*, 2011:815038.

12. Zintzaras, E., Papathanasiou, AA., Ziogas, DC., et al., (2012). The reporting quality of studies investigating the diagnostic accuracy of anti-CCP antibody in rheumatoid arthritis and its impact on diagnostic estimates. *BMC Musculoskelet Disord* 13: 113.

13. Liu, X., Jia, R., Zhao, J., et al., (2009). The role of anti-mutated citrullinated vimentin antibodies in the diagnosis of early rheumatoid arthritis. *J Rheumatol*, 36: 1136-42.

14. Poulsom H and PJ Charles (2008). Antibodies to citrullinated vimentin are a specific and sensitive marker for the diagnosis of rheumatoid arthritis. *Clin Rev Allergy Immunol* 34; 4-10.

15. Wagner, E., Skoumal, M., Bayer, PM., et al., (2009). Antibody against mutated citrullinated vimentin: a new sensitive marker in the diagnosis of rheumatoid arthritis. *Rheumatol Int*, 29: 1315-21.

16. Dejaco, C., Larcher, H., Duftner, C., et al., (2006). Diagnostic value of antibodies against a modified citrullinated vimentin in rheumatoid arthritis. *Arthritis Res Ther*, 8: R119.

17. Innala, L., Kokkonen, H., Eriksson, C., et al., (2008). Antibodies against mutated citrullinated vimentin are a better predictor of disease activity at 24 months in early rheumatoid arthritis than antibodies against cyclic citrullinated peptides. *J Rheumatol*, 35: 1002-8

18. Van Gestel, AM., Haagsma, CJ., van Riel, PL., (1998) Validation of rheumatoid arthritis improvement criteria that include simplified joint counts. *Arthritis Rheum*.;41:1845–50.

19. Prevoo, ML., van 't Hof, MA., Kuper, HH., et al. (1995). Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. *Arthritis Rheum*, 38: 44-8.

20. Hooi-Yeen, Yap., Sabrina Zi-Yi, Tee., Magdelyn Mei-Theng, Wong., et al., (2018). Pathogenic Role of Immune Cells in Rheumatoid Arthritis: Implications in Clinical Treatment and Biomarker Development; *Cells*: 7, 161.

21. Dorner, T., Egerer, K., and Feist, E., (2004). Rheumatoid factor revisited in RA. *Ann Rheum*; 55:186-190.

22. Nell, VPK., Machold, KP., Stamm, TA., et al., (2005). Autoantibody profiling as early diagnostic prognostic tool for rheumatoid arthritis. *Ann Rheum Dis*, 64: 1731-1736.

23. Khalifa, IA., Lotfy, AM., Elsayed, MA., et al., (2013). Anti-Mutated Citrullinated Vimentin Antibodies Rheumatoid Arthritis compared with anti-cyclic citrullinated peptides. *J Am Sci*, 9: 160-166.

24. Keskin, G., Inal, A., Keskin, D., et al., (2008). Diagnostic utility of anti-cyclic citrullinated peptide and anti-modified citrullinated vimentin antibodies in rheumatoid arthritis. *Protein Pept Lett*, 15: 314-7.

25. Morbach, H., Dannecker, H., Kerkau, T., et al., (2010). Prevalence of antibodies against mutated citrullinated vimentin and cyclic citrullinated peptide in children with juvenile idiopathic arthritis. *Clin Exp Rheumatol*, 28(5): 800-2.

26. Zendman, AJ., Venrooij, WJ van., and Pruijn, GJ., (2006). Use and significance of anti-CCP autoantibodies in rheumatoid arthritis. *Rheumatology* (Oxford), 45: 20-5.

27. El-Barbary, AM., Kassem, EM., El-Sergany, MA., et al., (2011). Association of anti-modified

citrullinated vimentin with subclinical atherosclerosis in early rheumatoid arthritis compared with anti-cyclic citrullinated peptide. *J Rheumatol*, 38; 828-34.

28. Barouta, G., Katsiari, CG., Alexiou, I., Liaskos, C., et al., (2017) Anti-MCV antibodies predict radiographic progression in Greek patients with very early (<3 months duration) rheumatoid arthritis. *Clin Rheumatol*, 36: 885-94.

29. Vittecoq, O., Incaurgarat, B., Jouen-Beades, F., et al., (2004). Auto antibodies recognizing citrullinated rat filaggrin in an ELISA using citrullinated and non-citrullinated recombinant proteins as antigens are highly diagnostic for rheumatoid arthritis. *Clin Exp Immunol*, 135: 173-80.19.

30. Mathsson, L., Mullazehi, M., Wick, MC., et al., (2008). Antibodies against citrullinated vimentin in rheumatoid arthritis: higher sensitivity and extended prognostic value concerning future radiographic progression as compared with antibodies against cyclic citrullinated peptides. *Arthritis Rheum*, 58: 36-45.

31. Nicaise Roland, P., Grootenboer Mignot, S., Bruns, A., et al., (2008). Antibodies to mutated citrullinated vimentin for diagnosing rheumatoid arthritis in anti-CCP-negative patients and for monitoring infliximab therapy. *Arthritis Res Ther*, 10: R142.

32. Kastbom, A., Forslind, K., Ernestam, S., et al. (2016) Changes in the anti-citrullinated peptide antibody response in relation to therapeutic outcome in early rheumatoid arthritis: results from the SWEFOT trial. *Ann Rheum Dis*, 75: 356-61.

33. Verpoort, KN., Cheung, K., A Ioan-Facsinay, AH van der Helm., et al., (2007). Fine specificity of the anti-citrullinated protein antibody response is influenced by the shared epitope alleles. *Arthritis Rheum*, 56:3949-52.

34. Bartoloni, E., Alunno, A., Bistoni, O., et al., (2012). Diagnostic value of anti-mutated citrullinated vimentin in comparison to anti-cyclic citrullinated peptide and anti-viral citrullinated peptide 2 antibodies in rheumatoid arthritis: an Italian multicentric study and review of the literature. *Autoimmun Rev*, 11: 815-20.

35. Al-Shukaili, A., Al-Ghafri, S., Al-Marhoobi, S., et al., (2012). Evaluation of anti-mutated citrullinated vimentin antibodies, anti-cyclic citrullinated Peptide antibodies and rheumatoid factor in Omani patients with rheumatoid arthritis. *Int J Rheumatol*, 2012:285854.

36. Sghiri, R., Bouajina, E., Bargaoui, D., et al., (2008). Value of anti-mutated citrullinated vimentin antibodies in diagnosing rheumatoid arthritis. *Rheumatol Int*, 29: 59-62.

37. Yousef ghahari, B., Alhooei, S., Soleimani-Amiri, MJ., et al., (2013). Comparison of sensitivity and specificity of anti-CCP and anti-MCV antibodies in an Iranian cohort of patients with rheumatoid arthritis. Caspian *J Intern Med*, 4: 702-6.

38. Ursum, J., Nielen, MM., van Schaardenburg, D., et al., (2008). Antibodies to mutated citrullinated vimentin and disease activity score in early arthritis: a cohort study. *Arthritis Res Ther*, 10: R12.

39. Coenen, D., Verschueren, P., Westhovens, R., et al., (2007). Technical and diagnostic performance of 6 assays for the measurement of citrullinated protein /peptide antibodies in the diagnosis of rheumatoid arthritis. *Clin Chem*, 53; 498-504.

40. Bang, H., Egerer, K., Gauliard, A., et al., (2007). Mutation and citrullination modifies vimentin to a novel autoantigen for rheumatoid arthritis. *Arthritis Rheum*, 56:2503-11.

41. Szekanecz, Z., Szabo,Z., Zeher, M., et al., (2013). Superior performance of the CCP3.1 test compared to CCP2 and MCV in the rheumatoid factor-negative RA population. *Immunol Res*, 56: 439-43.

42. Luime, JJ., Colin, EM., Hazes, JM., et al., (2010). Does anti-mutated citrullinated vimentin have additional value as a serological marker in the diagnostic and prognostic investigation of patients with rheumatoid arthritis? A systematic review. *Ann Rheum Dis*, 69: 337-44.

43. Mutlu, N., Bicakcigil, M., Tasan, DA., et al., (2009). Comparative performance analysis of 4 different anti-citrullinated protein assays in the diagnosis of rheumatoid arthritis. *J Rheumatol*, 36: 491-500.

44. Shidara, K., Inoue, E., Tanaka, D., et al.(2011). Comparison of the second and third generation anti-cyclic citrullinated peptide antibody assays in the diagnosis of Japanese patients with rheumatoid arthritis. *Rheumatol Int*, 31: 617-22.

45. Larsen, A., (1995). How to apply Larsen score in evaluating radiographs of rheumatoid arthritis in long-term studies. *J Rheumatol*, 22: 1974-5.

46. Saraux, A., Berthelot, JM., Devauchelle, V., et al., (2003). Value of antibodies to citrulline-containing peptides for diagnosing early rheumatoid arthritis. *J Rheumatol*, 30; 2535-9.

47. Whiting, PF., Smidt, N., Sterne, JA., et al., (2010). Systematic review: accuracy of anti–citrullinated peptide antibodies for diagnosing rheumatoid arthritis. *Annals Of Internal Medicine*, 152: 456-64.

48. Sun, J., Zhang, Y., Liu, L., et al., (2014). Diagnostic accuracy of combined tests of anti-cyclic citrullinated peptide antibody and rheumatoid factor for

rheumatoid arthritis: a meta-analysis. *Clin Exp Rheumatol:* 32; 11-21.

49. El-Badawy, MA., El-Mahdi, AR., El Rehem, SMA., et al., (2017). Evaluation of disease activity markers in relation to dry eye disease in patients with rheumatoid arthritis. *Egyptian Rheumatology and Rehabilitation,* 44: 111.

50. Engelmann, R., Brandt, J., Eggert, M., et al., (2009). The anti-mutated citrullinated vimentin response classifies patients with rheumatoid arthritis into broad and narrow responders. *J Rheumatol,* 36:2670-4.

51. Harre, U., Georgess, D., Bang, H., et al. (2012). Induction of osteoclastogenesis and bone loss by human autoantibodies against citrullinated vimentin. *J Clin Invest,* 122: 1791-802.