Research Article

Anti-Carbamylated antibodies as a marker of activity and disability in Rheumatoid Arthritis

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Abstract
Objective: To investigate whether serum levels of anti-Carbamylated antibodies are specifically elevated in active rheumatoid arthritis compared to clinical and laboratory markers of disease activity. **Methods**: Sixty rheumatoid arthritis patients according to the ACR-EULAR criteria 2010 and 30 matched healthy controls were recruited. Clinical evaluation of disease activity was measured by; morning stiffness duration, Visual analogue scale, number of tender joints, number of swollen joints and DAS-28. Functional assessment was measured by the health assessment questionnaire (HAQ). Laboratory measures; ESR, CRP and RF titer was also done and finally radiological activity by ultrasound 7 score were determined parallel and correlated. **Results**: anti-Carbamylated antibody was found to be positive in more than 50% of patients with active RA. Those patients were seronegative for RF. Anti-Carbamylated antibody positivity was significantly associated with duration of morning stiffness, number of tender joints, number of swollen joints, DAS28, HAQ DI, and acute phase reactants (ESR & CRP). **Conclusion:** Anti-CarP antibody was found to be positive in RA patients and it was significantly correlated with clinical, laboratory and radiological markers of disease activity as well as the functional assessment.
Keywords: anti-Carbamylated, antibodies, rheumatoid arthritis

Introduction
Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease, characterized by synovitis, bone and cartilage damage, systemic inflammation and generation of autoantibodies[1].
The 2015 American College of Rheumatology (ACR) recommends treat to target[2]. In order to be adherent to these recommendations, regular assessment of disease activity is recommended in routine care[3]. Different clinical activity measures were recommended; the Clinical Disease Activity Index (CDAI), Disease Activity Score in 28 joints (DAS28), Patient Activity Scale (PAS), Patient Activity Scale II (PAS-II), Routine Assessment of Patient Index Data3 (RAPID3), and Simplified Disease Activity Index (SDAI)[4].

There are some limitations regarding the use of these scores like musculoskeletal ultrasound or magnetic resonance imaging[3]. Also these scores which include patient reported measures may show a higher activity measures in subpopulation of RA with comorbid fibromyalgia[5]. RA patients with associated high body mass index may show a higher inflammatory markers[6]. Providing recommendations for disease activity assessment in such specific situations are left to the treating physician[3].
Diagnosis and management of RA depends mainly on the RF and ACPA, but the clinical area still in need for more serological makers[7]. Recent autoantibodies has been described which react against Carbamylated proteins (anti-Car-P) carbamylation as citrullination are post-translational modifications which end in the production of homocitrulline and citrulline respectively.

Anti-Car-P antibodies were found in RA patients[8]. Interestingly they are detected in patients negative for anti-CCP[9]**.** Whereas citrullinated proteins have been identified in the joints of RA patients, the presence of Carbamylated proteins has not been clearly demonstrated[10]. However, indirect evidence has been strongly observed, notably through the detection of anti-carbamylated protein (Car-P) antibodies[11]. The presence of anti-Car-P antibodies has been described in several cohorts of RA patients[12-16]. Anti-Car-P antibodies may also be present prior to onset and predict the evolution of RA, which has been shown in some studies[9,17,18]. The presence of anti-Car-P

antibodies was reported to be associated with a severe disease course characterized by rapid radiological progression[11]. Anti-Car-P antibody was assessed in psoriatic arthritis patients, it was suggested that may represent a promising biomarker to predict joint damage and disease activity[19].

Patients and Methods

Sixty patients with established rheumatoid arthritis, according to the ACR-EULAR criteria 2010[20], were recruited from the outpatient clinic of Rheumatology department during the period from November 2018 to July 2019. The followings were excluded from the study; smokers, patients with kidney diseases, malignancies and viral hepatitis. Thirty healthy volunteers' age and sex matching were considered as a control group. An oral consent was obtained from all participants and was approved by the university research ethics committee.

All patients were subjected to detailed history taking, full clinical examination, assessment of tender and swollen joints, pain by visual analogue scale (VAS), disease activity score (DAS28)[21], of the health assessment questionnaire (HAQ)[22]. Radiological assessment was done by the US 7 for accurate assessment of synovitis, tenosynovitis, synovitis and tenosynovitis with positive Doppler signals[23].
Laboratory investigations included complete blood count (CBC), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and rheumatoid factor (RF). Anti-carbamylated protein antibodies were detected in serum by Human anti-carbamylated protein Antibody ELISA kit.

Disease activity
DAS28 was used to evaluate disease activity. Patients scored their pain using the visual analogue scale (VAS), ranging from 0 (no pain) to 100 (the worst pain imaginable). The counts for tender and swollen joints and erythrocyte sedimentation rates were also recorded. Patients were categorized according to the DAS28 results as high disease activity (>5.1), moderate disease activity (3.2–5.1), low disease activity (2.6–3.2), and remission (< 2.6).

Statistical analysis: SPSS for windows version 19.0 was used to perform the statistical analyses. Data were tested for normality of distribution. Data were expressed as mean ± standard deviation (SD) or number and percentage and median and interquartile range (IQR. The comparisons were determined using Student's t test or Mann-Whitney U test for variables with continuous data of normal and abnormal distribution respectively. Chi-square test was used for comparing variables with categorical data. And Correlations were determined by Pearson and Spearman correlation coefficients.

Results

The studied sixty RA patients included 53 females (88.3%) and 7 males (11.7%) with their ages ranged from 24 to 64 years with a mean of 47.32±9.5 and duration of illness ranged from 1 to 25 years with a mean of 6.35±5.88. The control group included 30 healthy volunteers 22 females (73.3%) and 8 males (26.7%); their age ranged from 27 to 60 years with a mean of 45.50±8.7 year table 1. The clinical and laboratory data of RA patients are presented in table 2

Table (1): Demographic data of RA patients and control group

		patients	control	p
Age		47.3±9.5	45.5±8.7	o.46
Gender	Male (n, %)	7 (11.7%)	8 (26.7%)	0.70
	Female (n, %)	53 (88.3%)	22 (73.3%)	

Independent sample t-test and chi square, significant p value <0.05

Comparison between patients with seropositive and seronegative anti- Car-P showed a statistically significant difference regarding duration of morning stiffness, VAS, number of tender joints, number of swollen joints and HAQ-DI. Also there was a statistically significant difference regarding the laboratory results; ESR, CRP and RF titer. Finally DAS 28 was also of significant statistical values. Interestingly there was no correlation to be

found between anti-CarP and disease duration as shown in table 3

Regarding ultrasound evaluation; the comparison was statistically significant specifically synovitis (gray scale and Doppler signals) and tenosynovitis in its Doppler findings only Table 4

Anti-CarP positive RA patients showed a strong positive correlation with statistically significant values regarding disease activity measures (VAS, number of swollen joints, synovitis in gray scale, ESR, CRP and DAS 28). There were also moderate correlations with statistically significant values regarding duration of morning stiffness, number of tender joints, HAQ, synovitis and tenosynovitis regarding their Doppler views table 5.

Table (2): The clinical, laboratory and ultrasound data of RA patients

	Mean± SD
Morning stiffness (minutes)	40.2±38.3
28 tender joint count	5.9±4.5
28 swollen joint count	4.1±4.3
VAS	4.3±2.2
DAS 28	4.1±1.3
HAQ-DI	1.7±1.0
ESR 1st hour (mm/h)	32.7±18.7
CRP	7.2±2.9
RF	138.8±241.1
Anti CCP positive (n, %)	30 (50%)
Anti-carp positive (n, %)	28 (46.7%)
Ultrasound 7 score	
Synovitis in GSUS	13.45±7.05
Synovitis with positive Doppler signals	5.12± 5.9
Tenosynovitis in GSUS	2.43± 1.98
Tenosynovitis with positive Doppler signals	2. 38±3.40

VAS; visual analogue scale, DAS28; disease activity score 28 joint count, HAQ DI; health assessment questionnaire disease index, ESR; erythrocyte sedimentation rate, CRP; C reactive protein, RF; Rheumatoid factor, Anti CCP; anti citrullinated peptide, anti Crap; anti carbamylated protein, GSUS; Grey Scale Ultrasound, PDUS; Power Doppler Ultrasound

Table (3): comparison of clinical data between subgroups

	Anti-Car-P positive (N=28)	**Anti-Car-P negative (N=32)**	**t**	**P**
Disease duration	5.74±5.26	9.4±6.96	1.82	0.072
Morning stiffness duration (minutes)	53.89 ± 42.99	28.28 ± 5.21	2.72	**0.009**
VAS	5.82 ± 1.66	3.00 ± 1.78	6.33	**0.000**
HAQ DI	2.21 ± 0.74	1.28 ± 1.02	4	**0.000**
Tender joint count	7.64 ± 4.31	4.28 ± 4.28	3.12	**0.003**
Swollen joint count	6.96 ± 3.86	1.66 ± 2.84	6.12	**0.000**
ESR	47.18 ±14.75	20.03 ±10.86	8.18	**0.000**
CRP	8.98 ±1.43	5.69 ±3.06	5.21	**0.000**
RF	234.57± 312.84	55 ± 99.14	3.08	**0.003**
DAS 28	5.239 ±.98	3.538 ± 1.24	5.82	**0.000**

By independent sample t test; VAS; visual analogue scale, HAQ DI; health assessment questionnaire disease index, ESR; erythrocyte sedimentation rate, CRP; C reactive protein, RF; Rheumatoid factor, DAS28; disease activity score 28 joint count, Significant p-value<0.05

Table (4): Comparison between patient's groups regarding Ultrasound 7 score

	Anti-Car-P positive (N=28)	Anti-Car-P negative (N=32)	t	P value
Synovitis in GSUS (Mean ± SD)	9.78 ± 6.36	17.64 ± 5.27	5.2	**0.001**
synovitis with positive Doppler signals (Mean ± SD)	3.0± 3.44	7.54 ± 6.77	3.3	**0.01**
Tenosynovitis in GSUS (Mean ± SD)	1.94 ± 1.63	2.93 ± 2.24	2	**0.053**
Tenosynovitis with positive Doppler signals (Mean ±SD)	1.16 ± 2.75	3.79 ± 3.56	3.2	**0.002**

*By independent sample t-test, * Significant P value < 0.05, **High significant P value < 0.001, GSUS; Grey Scale Ultrasound, PDUS; power Doppler ultrasound*

Table (5) correlation between Anti- CarP and disease activity measures

	anti-CarP	
	r	**P**
Duration of morning stiffness	0.36	**0.005***
VAS	0.65	**0.001****
HAQ-DI	0.46	**0.001****
Tender joint count	0.46	**0.001****
Swollen joint count	0.69	**0.001****
ESR	0.62	**0.001****
CRP	0.39	**0.002***
DAS 28	0.63	**0.001****
Synovitis in GSUS	0.56	**0.001****
synovitis with positive Doppler signals	0.43	**0.001****
Tenosynovitis in GSUS	0.21	0.1
Tenosynovitis with positive Doppler signals	0.49	**0.001****

** Significant p value <0.05, ** High significant P value < 0.001.*

Discussion

There is no doubt that Rheumatoid factor and anti citrullinated antibodies are important makers for the diagnosis of RA. Although ACPA have significantly improved its diagnosis but it is unquestionable that there is a demand for a novel antibodies to add a more diagnostic value as well as to stratify patients according to different disease phenotype (activity and treatment response)[24, 25]. After the era of DMARDs in treatment of RA, the prediction of disease activity and severity

became more and more important as its early and aggressive use can prevent irreversible joint damage and disability[7]. This study was to evaluate the anti-carp in patients with RA and its relation to disease activity as well as functional assessment. Our results have shown that RA patients with sero positivity of anti-Carp antibodies have more active disease and they were more disabled compared with sero negative patients. we reported a significant association between anti-CarP positivity and DAS-ESR levels in agreement with truchete and his colleagues on 2017 who find a significant association between the anti-CarP anti-bodies and severe disease clinical activity[26]. Similar data could be recorded from a study by Othman et al., on 2017 who reported a significant association between anti-CarP level and HAQ and CRP[27]. Also similar results were reported by Humphreys and colleagues in 2016[28]. We found a significant moderate correlation between Anti-CarP and DAS 28 similar to data reported by Yee et al., on 2015[7]; they found a significant mild correlation with DAS28. Another study by Sahar et al., on 2019 who found same moderate significant correlation[29]. In our study, we reported that patients with anti-CarP-antibodies were more disabled than those with anti-CarP-negative. This was explained by the significant difference between patients with positive and patients with negative anti-CarP antibodies and the HAQ results. Our finding is almost in agreement with other previously reported studies[28,30]. Also there was a significant association between anti-CarP and HAQ results demonstrated by Othman et al., on 2017[27]. Our data reported no correlation between anti-CarP antibodies and disease duration in agreement with same findings of other study[7]

In Conclusion, the results obtained demonstrate an association between anti-CarP antibodies positivity and the increased disease activity. It could be useful to test beside other known disease activity measures and could be considered as a predicative measure for disability.

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