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ORIGINAL ARTICLE

Diagnostic and prognostic value of anti-CarP antibodies in a sample of Egyptian rheumatoid arthritis patients

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Abstract

Introduction Detection of autoantibodies in sera of rheumatoid arthritis (RA) patients has an important role in diagnosis and management strategies. Recently, another type of autoantibodies has been detected with activity against carbamylated proteins (anti-CarP) which may play an important role in the diagnosis of RA. The aim of this study was to raise knowledge about the diagnostic and prognostic value of anti-CarP antibodies in RA.

Materials and methods Seventy RA patients and thirty-four controls were included in this study. DAS28 was used to evaluate disease activity. Joint erosions were assessed by Larsen score using plain X-ray of involved joints of hands and feet. Serum samples were analyzed for anti-CarP antibody titer using the ELISA technique.

Results Out of 70 patients, 35.7% were positive for anti-CarP and only 5.88% of controls had high titer above the cut-off value. A total of 24.29% of the patients were RF-negative and 30% were ACPA-negative. Five patients (29.41%) of the negative RF group were positive for anti-CarP. Four patients (19%) of the ACPA-negative group were positive for anti-CarP, and three patients (4.28%) of the total number of patients were triple negative and seventeen (24.28%) were triple positive. There was a significant correlation between anti-CarP titer and both DAS28 and Larsen scores only in the positive anti-CarP group. In addition, there was a strong association between anti-CarP antibody titer and joint erosions at both baseline and after 1-year follow-up.

Conclusion Presence of the anti-CarP antibodies in sera of RA patients may have a prognostic value as it correlates with the disease activity and joint erosions; moreover, it may have a diagnostic value in rheumatoid arthritis especially in RF- and ACPA-negative patients.

Key Points
- *This study was carried out to raise our knowledge about the importance of anti-CarP antibodies in predicting the prognosis of RA.*
- *This study was carried out to assess the correlation between anti-CarP antibodies, disease activity, and joint erosions.*
- *This study was carried out to state the extent to which we can rely on the anti-CarP antibodies as a biomarker for prediction of RA.*

Keywords Anti-CarP antibodies in rheumatoid arthritis · Assessment of disease activity · Relation of autoantibodies with joint erosions

Introduction

Rheumatoid arthritis (RA) is an autoimmune inflammatory disease with unknown etiology [1]. It is commonly presented by symmetric polyarthritis that may or may not be associated with extra-articular manifestations [2]. In developed countries, the prevalence ranges between 0.3 and 1% [3]. Uncontrolled cases of RA may develop joint erosions and deformities [4]. Theories that explain the development of RA suggest genetic and environmental factors [5, 6]. Prevalence of RA is higher in females as compared with males. RA patients may present with pain, fatigue, decreased range of motion, muscle weakness, and stiffness which may lead to disability, decreased quality of life, and high mortality [1, 7, 8]. Nowadays, advances in the treatment including new therapies and treatment strategies had led to decreased disease activity and improved rate of remission [9, 10].

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Detection of autoantibodies in RA patients has an important role in understanding disease process and patients' classification [1]. The two common autoantibodies in RA patients are the rheumatoid factor (RF) and anti-CCP antibodies [11]. For decades, RF was the only autoantibody detected in patients with RA but later anti-citrullinated protein antibodies were discovered and it is found to be highly specific for RA [12]. The main RF species in RA is IgM-RF and it is found in up to 60–80% of patients. However, RF may be found in other autoimmune diseases such as primary Sjogren syndrome and systemic lupus erythematosus [13] and in chronic infections in addition to old age [14]. High titer of RF increases the possibility of RA and may be associated with increased incidence of extra-articular manifestations, joint erosions, and bad prognosis [15–17].

Autoantibodies including RF and ACPA have an important role in 2010 RA classification criteria as they account for up to 3 of the 6 points needed for RA classification [18, 19]. ACPA is present in 70–90% of RA patients and its sensitivity ranges from 90 to 95%, and it is very rare to be present in other diseases and healthy people [6, 20, 21]. ACPA has higher sensitivity and specificity for RA than RF, and its presence may be associated with increased incidence of joint erosions. Furthermore, ACPA may help in predicting disease activity and play a role in the prognosis of RA [18].

Carbamylation is a post-translational process of proteins via cyanate. The carbamylation level is directly proportional with the urea level and inversely with the level of the free amino acids which protect protein from carbamylation. Carbamylation may alter both structure and function of a protein and may implicate in many diseases and inflammation. In addition, it may be used as a risk assessment for certain diseases such as kidney diseases. Since there is a link between carbamylation and disease pathophysiology, then reducing the rate of carbamylation is considered a promising therapeutic target [22]. Recently, autoantibodies for carbamylated proteins (anti-CarP) have been detected [23]. Like the presence of ACPA and RF antibodies before the clinical symptoms of RA, anti-CarP have been detected in the sera of healthy individuals before the onset of RA [24] and might represent a promising marker to predict joint damage in RA [25, 26]. This study was carried out to raise our knowledge about the importance of anti-CarP antibodies in predicting the prognosis of RA by assessing the correlation between anti-CarP antibodies, disease activity, and joint erosions and to declare the extent to which we can rely on the anti-CarP antibodies as a biomarker for prediction of RA.

Materials and methods

Seventy RA patients were selected from the outpatient clinic of rheumatology in Sohag University Hospital, Egypt, in the period from March 2017 to September 2018, and thirty-four age- and gender-matched healthy volunteers were included as controls. Informed consents were collected from all patients and ethical permission was obtained from the ethical committee of Sohag University. Rheumatoid arthritis patients were diagnosed according to the American College of Rheumatology 2010 criteria. Patients with other connective tissue diseases such as systemic lupus erythematosus and scleroderma or non-consenting were excluded. Blood samples were collected from all patients, and complete blood count, erythrocyte sedimentation rate, rheumatoid factor, and ACPA were assayed. Serum samples were analyzed for anti-CarP antibody titer using an enzyme-linked immunosorbent assay (ELISA) kit (SinoGenecion, China) according to the included protocol.

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 recorded. According to the DAS28 score, disease activity in RA patients was categorized as high disease activity (> 5.1), moderate disease activity (3.2–5.1), low disease activity (2.6–3.2), and remission (< 2.6).

Assessment of joint erosions

Joint erosions were assessed by Larsen score using plain X-ray of involved joints of hands and feet; Larsen's scoring system attributes 0 to 5 points to each synovial joint evaluated on radiographs of hands, wrists, and feet with a maximum possible score of 250 [27].

Statistical analysis

Baseline characteristics between controls and patients were compared using Microsoft Excel and SPSS (version 17). The correlation study among the different parameters performed using Pearson's coefficient of correlation (r value). $p < 0.05$ was considered statistically significant.

Results

Seventy RA patients and thirty-four controls were included in this study. Demographic parameters of patients and controls are shown in Table 1. The calculated cut-off value of the anti-CarP antibody was 1.78 ng/ml. Twenty-five out of seventy (35.7%) of RA patients had a high titer of anti-CarP antibodies above the cut-off value. On the other hand, only two out of thirty-four (5.88%) of controls had a high titer of anti-CarP

Table 1 Demographic parameters of patients and controls

	Patients	Control
Age (mean ± SD)	46.98 ± 10.95	46.16 ± 10.12
Gender (male/female)	13/57	3/13
BP (systolic mmHg)	121.42 ± 3.21	123.12 ± 3.87
BP (diastolic mmHg)	81.62 ± 2.98	80.25 ± 3.17
ESR	41.92 ± 19.91	8.53 ± 2.1
RF	64.51 ± 53.24	10.1 ± 4.21
Anti-CarP (ng/ml)	2.98 ± 0.369	0.836 ± 0.122

antibodies above the cut-off value. There was a significant difference ($p < 0.01$) in the mean value of anti-CarP antibodies between patients and controls (Fig. 1a). Fifty-three (75.71%) patients were RF-positive and seventeen (24.28%) were RF-negative. Concerning ACPA reactivity, forty-nine (70%) were positive and twenty-one (30%) were ACPA-negative (Fig. 1b). Five patients (29.41%) of the negative RF group were positive for anti-CarP. Four patients (19%) of the ACPA-negative group were positive for anti-CarP, and three patients (4.28%) of the total number of patients were triple negative. Of the total patients, seventeen (24.28%) were triple positive. Of our small sample size, four patients were ACPA-negative anti-CarP-positive and 19 patients were ACPA-negative anti-CarP-negative; because the two groups have an unequal sample size, so statistical power and type 1 error rates will be dramatically affected. The patients were divided into two groups according to reactivity to anti-CarP antibodies; patients who have anti-CarP titer above the cut-off value have been considered positive and those who have titer less than the cut-off value have been considered negative. There was a significant ($p < 0.001$, $p < 0.05$, $p < 0.05$) difference between the mean of anti-CarP, DAS28, and Larsen between the positive and negative groups, but there was no significant difference in both RF and ESR (Fig. 2); moreover paired t test revealed a significant ($p < 0.05$) difference in the Larsen score after 1 year from the baseline in the positive patients only (Fig. 2).

Correlation of anti-CarP antibodies with disease activity and joint erosions

We tested the correlation between disease activity and joint erosions in the positive and negative groups (Table 2). Pearson's correlation coefficient revealed a significant positive correlation between the values of DAS28 and the titer of anti-CarP antibodies in the positive patients ($r = 0.527$, $p < 0.01$) (Fig. 3); however, we could not find any correlation in the negative group. The mean Larsen score of anti-CarP antibody–positive patients was 64.41 with a standard deviation of 32.22 and 49.71 with a standard deviation of 15.24 for negative patients. There was a significant ($p < 0.05$, $p < 0.001$) difference in the Larsen score at baseline and after 1 year between positive and negative groups (Fig. 2). The joint erosions were significantly higher in anti-CarP-positive patients in comparison to the negative patients. There was a strong association between anti-CarP antibody titer and joint erosions at both baseline and after 1 year ($r = 0.460$; 95% CI, 0.07921 to 0.7237; $p < 0.05$ and $r = 0.408$; 95% CI, 0.01543 to 0.6918; $p < 0.05$, respectively). On the other hand, there was no significant correlation between anti-CarP antibody titer and joint erosions in the anti-CarP-negative patients (Fig. 3).

Discussion

Detection of autoantibodies as ACPA and RF in sera of rheumatoid arthritis patients is important in the classification of the patients and plays a vital role in anticipating prognosis and management strategies [4, 6]. Recently, other autoantibodies have been discovered as anti-CarP antibodies [23, 26], which

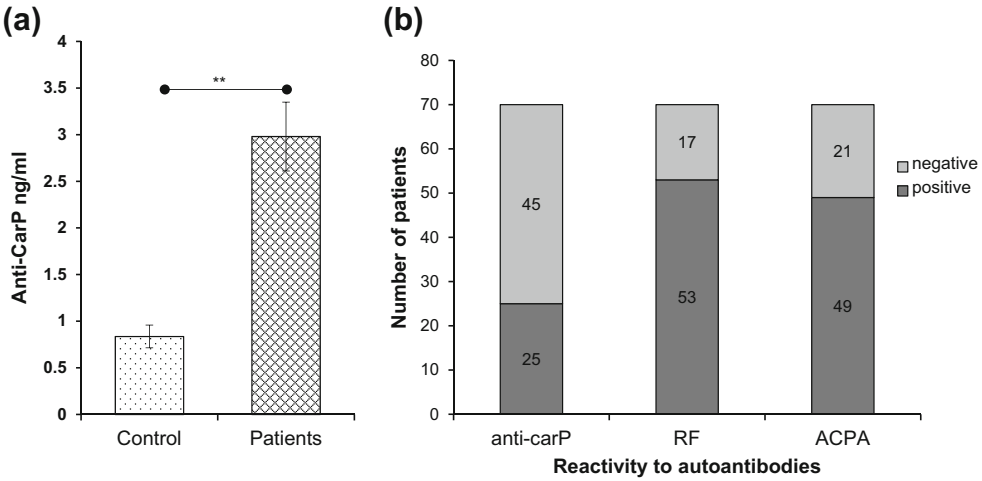

Fig. 1 Anti-CarP antibody titer in the studied groups. **a** Comparison of anti-CarP antibody titer in patients and controls. Values are mean ± SEM and ** at $p < 0.01$. **b** Reactivity of patients to autoantibodies. The number in each segment of columns represents the positive or negative patients out of seventy

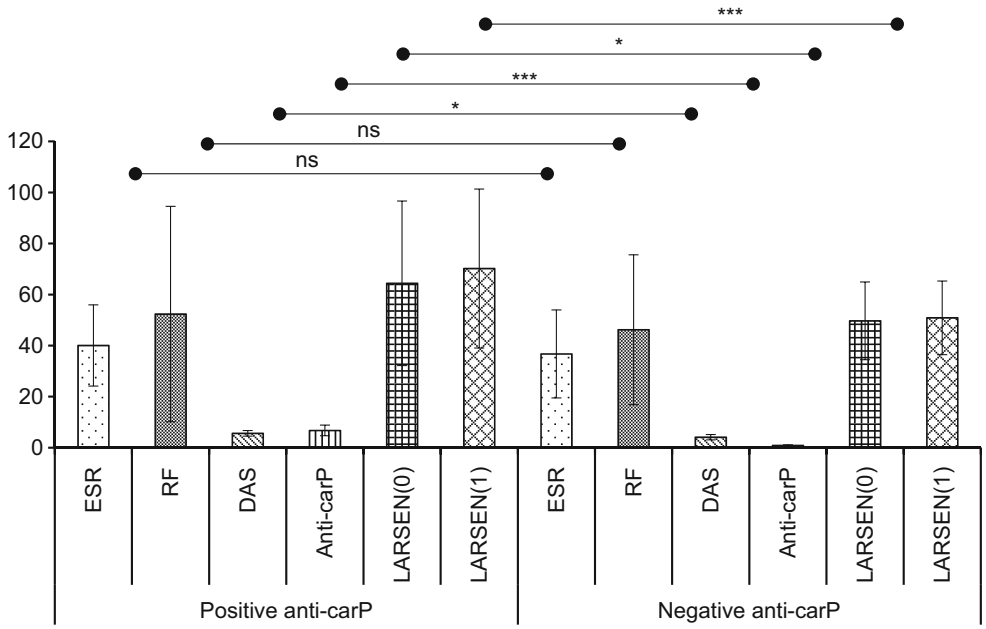

Fig. 2 Comparison of the mean values of the studied parameters ± the SD in the positive and negative groups, where * means $p < 0.05$ and *** means $p < 0.001$

were first discovered in patients with arthralgia [26] but now have been found in rheumatoid patients either ACPA-positive or ACPA-negative individuals [25]. In our study, 35.7% of RA patients had a high titer of anti-CarP antibodies above the cut-off value and have been a positive group for anti-CarP antibodies. Our finding is almost in agreement with other previously reported studies [25, 26, 28]. Studying the correlation between serum anti-CarP antibodies and disease activity or any other parameter revealed a strong positive correlation between the anti-CarP titer and DAS28 score in agreement with Kumar et al. [29]; however, de Moel et al. reported that anti-CarP are not associated with DAS28 but just reflect intensity of immunosuppression [30]. Significant correlation between DAS28 and Larsen score is in agreement with Yee et al. [25]; also, there was a correlation between duration and Larsen score but there was no correlation between DAS and RF of the positive group; on the other hand, we could not find any correlation between any of these parameters in the negative group. There was a significant association between serum titer of anti-CarP antibodies and joint erosions. Our finding is in agreement with the finding of Ajeganova et al. [28]. This may explain the ongoing joint damage with the high serum titer of anti-CarP antibodies, and this was clear when we compared Larsen score at baseline and after 1 year in the positive anti-CarP group.

Table 2 Correlation between the anti-CarP titer with rheumatoid factor, ACPA, DAS28, and Larsen in anti-CarP-positive patients. $p < 0.05$ is considered significantly different

Item	*r*	*p* value
anti-CarP vs RF	0.197	ns
anti-CarP vs ACPA	0.04	ns
anti-CarP vs DAS28	0.527	$p < 0.01$
anti-CarP vs Larsen(0)	0.46	$p < 0.05$
anti-CarP vs Larsen(1)	0.40	$p < 0.05$

Carbamylation is a post-translational modification of amino acids or proteins. Isocyanic acid, the main player of carbamylation, arises from two major pathways inside the living organisms; one of these pathways is non-enzymatic modification between isocyanic acid and N-terminal of lysine amino acid, and the second pathway is from oxidation of thiocyanate by myeloperoxidase in the presence of H_2O_2 in the inflamed sites [31]. Isocyanic acid is present at very low concentration in the living organisms and produced from decomposition of urea, so high level of urea can reflect its concentration [32, 33], and thiocyanate is found in high levels in smokers [34] and also in atherosclerotic plaques [35]. Carbamylation of proteins is implicated in many pathological situations. Carbamylation of lipoproteins can largely affect its functions; for instance, Carbamylation of LDL induces endothelial dysfunction and carbamylation of HDL leads to endothelial cell death, so carbamylation may be implicated in atherogenesis [36]. Recently, other recent reports emerged which consider carbamylation of proteins as one of the risk factors for cardiovascular diseases [37]. Carbamylated protein can elicit the immune system and augment inflammation, and this may explain the positive correlation between high serum titer of anti-CarP, disease activity, and joint erosions.

Presence of autoantibodies before the onset of a disease may contribute to pathogenesis, for instance, secretion of the inflammatory mediators and complement activation; and its

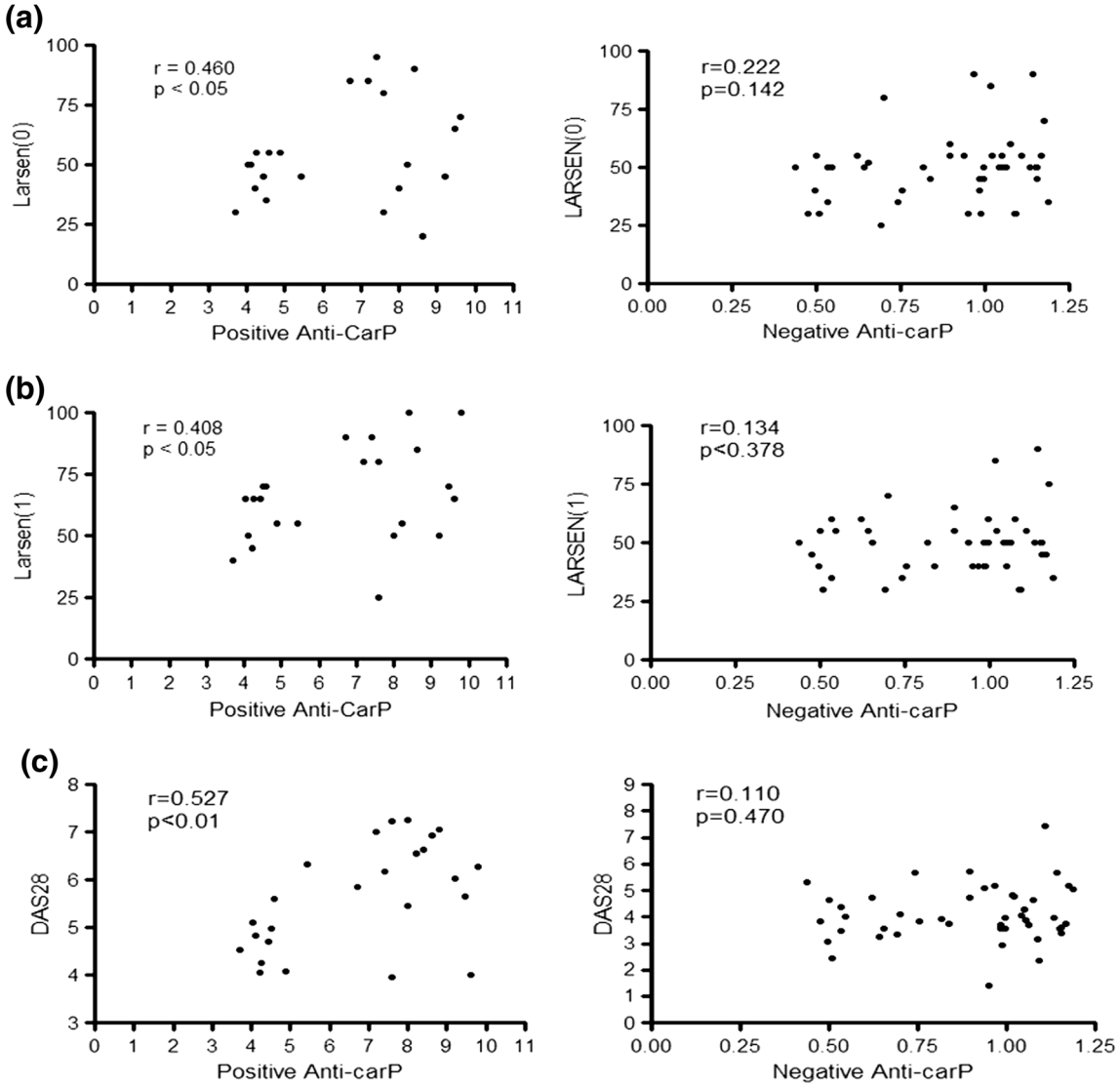

Fig. 3 Correlation of anti-CarP with disease activity and joint erosions. **a** Larsen score with anti-CarP in positive and negative patients at baseline. **b** Larsen score with anti-CarP in positive and negative patients after 1 year. **c** DAS28 score with anti-CarP in positive and negative patients

detection before the appearance of the clinical symptoms may be of clinical significance value especially if it is specific for a certain disease. If anti-CarP antibodies have an appropriate sensitivity for RA, then its detection before the onset of the disease will add to the diagnosis especially in patients who are RF- and ACPA-negative. Our finding supports this hypothesis as we found that 29.41% of the RF-negative group were positive for anti-CarP antibodies and 19% of the ACPA-negative group showed positivity for anti-CarP antibodies. This means that adding anti-CarP antibodies to the known autoantibodies (RF and ACPA) may help in the diagnosis of patients previously classified as seronegative. Recently, Regueiro et al. have found a specific association of HLA–DRB1*03 with ACPA −/anti-CarP+RA, suggesting that preferential presentation of carbamylated peptides could be a new mechanism underlying the contribution of HLA alleles to RA susceptibility [38]. In conclusion, the presence of the anti-CarP antibodies in sera of RA patients may have a prognostic value as it correlates with the disease activity and joint erosions; moreover, it may have a diagnostic value in rheumatoid arthritis especially in RF- and ACPA-negative patients. However, still, some patients are triple seronegative which necessitates searching for other autoantibodies.

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Authors' contributions This study was conceptualized and designed by SAE, MAI, RMA, and OMM. Patients were selected and diagnosed by SAE, MAI, and RMA. Data sheets were written by RMA. Lab assessments were done by OMM. All authors interpreted the data and discussed the results, and the manuscript was written and revised by SAE.

Compliance with ethical standards

Informed consents were collected from all patients and ethical permission was obtained from the ethical committee of Sohag University.

Disclosures None.

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