Egyptian Rheumatology and Rehabilitation

RESEARCH Open Access

Do anti-carbamylated protein antibodies in rheumatoid arthritis reflect local and systemic osteoporosis? A study of osteoprotegrin and receptor activator for nuclear factor kappa B ligand and radiological assessment

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Abstract

Background: One of the most important and dangerous complications of rheumatoid arthritis (RA) is bone loss, which manifested by erosions and juxta-articular or systemic bone loss. Anti-carbamylated protein (anti-CarP) antibodies which are also called anti-homocitrulline antibodies have recently been found in RA. Increase anti-CarP antibody titres may lead to severe disease and increase the progression of bone loss. Osteoprotegrin and receptor activator for nuclear factor kappa B and its ligand (RANKL) are the main players in the pathogenesis of osteoporosis. Thus, we aimed to investigate and detect the presence and prevalence of anti-CarP in rheumatoid arthritis and their association with disease severity and osteoporosis, as well as with OPG/RANKL in 80 Egyptian RA patients to highlight this relationship which could be useful in managing RA patients with osteoporosis.

Results: Serum anti-CarP levels were significantly increased in the RA group compared with the control group ($P<0.001$). We found a negative association between anti-CarP and anti-CCP and disease activity ($r=-0.878, -0.534$, respectively, $P<0.001$). We also found a positive correlation between anti-CarP and the Larsen score, DEXA score, RF, HAQ, and RANKL ($r=0.646, 0.287, 0.243, 0.892, 0.671, 0.869$ [respectively], $P<0.001$) and there was negative correlation between anti-CarP and OPG ($r=-0.553$, $P<0.001$).

Conclusion: Anti-CarP antibodies are associated with disease severity and disability in RA patients. They could play an important and significant role in the pathogenesis of osteoporosis in these patients.

Keywords: Rheumatoid, Osteoporosis, Anti-carbamylated antibodies, Osteoprotegrin, RANKL

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Background

Rheumatoid arthritis is a chronic autoimmune inflammatory disease that is manifested by synovitis and joint destruction [1]. Erosion and bone loss (juxta-articular or systemic) are prevalent [2].

Erosion usually occurs at the bone surface beneath the inflamed tendon and synovium and it is defined as focal loss of bone (cortical and trabecular) [3]. RA patients have higher risk of osteoporosis than normal subjects. This mostly occurs in the lumbar spine (LS) and hip region [4, 5]; and there is a high risk of fractured hip and spine depending on duration of the disease and medications that are used [6–9].

The increased risk of osteoporosis in RA patients can be caused by many factors such as corticosteroid medications that are taken for the disease. Decreased activity due to pain may also increase the risk of osteoporosis; and this risk may also increase due to disease progression [10].

In the complex system of bone remodeling, RANKL/OPG pathway is the coupling factor between bone formation and bone resorption. RANKL acts through binding to its receptors on the surface of osteoclasts and activates differentiation of these cells. Also, the balance between OPG and RANKL determines osteoblast proliferation and activity, and OPG binding to RANKL lead to inhibition of osteoclastic bone resorption [11].

Anti-carbamylated protein (anti-CarP) antibodies are the most recent antibodies that have been detected in RA. Increase anti-CarP antibody titres may lead to severe disease and increase the progression of bone loss. Even within ACPA-negative patients, carbamylation is a process in which a cyanate group is added on self-proteins to determine the changes in the tertiary structure. This can lead to new epitope generation and production of autoantibodies. Additionally, anti-CarP antibodies seem to play an important pathogenic role in RA which is similar to ACPA. Anti-CarP antibodies may be found in the serum for a long time before disease manifestations appearance [12].

The aim of our study was to investigate the prevalence of anti-CarP in RA, its correlation and its association with the severity of the disease and osteoporosis as well as with OPG/RANKL. Thus, these antibodies could be very useful in managing of RA patients with osteoporosis.

Methods

This was a cross-sectional study in which 80 RA patients were selected randomly and forty healthy controls that were matched for age and sex were included for laboratory investigations.

The patients and control subjects were chosen from the inpatient unit and outpatient clinic in the Rheumatology and Rehabilitation Department at our University Hospital from October 2019 to February 2020.

Inclusion criteria

We included all patients with RA who were diagnosed according to the American College of Rheumatology (ACR)/European League against Rheumatism (EULAR) 2010 criteria for the diagnosis of RA [13].

Exclusion criteria

We excluded RA patients who were taking anti-resorptive drugs, patients with other inflammatory arthritic diseases, and those receiving corticosteroids or other medications that affect bone remodeling.

Written informed consent was obtained from the patients and controls at the beginning of the study, and our study was approved by the ethics committee at our university (approval code 34206-10-19) and it was conducted in accordance with the Declaration of Helsinki. All patients provided a detailed history and underwent full clinical examinations. We used a predesigned and validated questionnaire sheet that included the following: sociodemographic data such as sex, age, and disease duration, measures of disease activity using disease activity score 28 (DAS28) [14], Health Assessment Questionnaire (HAQ) [15], and plain X-ray of hands and feet for assessment of radiographic damage by a modified Larsen's score [16].

Laboratory assessments

Blood sampling

After 12 h of overnight fasting, venous blood samples were obtained from our patients and controls. Some of the blood was collected in centrifuge tubes that were sterile and dry. After allowing the blood to clot, it was centrifuged for 10 min, and the serum was stored frozen at −80 °C after collection until analysis. Another sample was collected into heparinized tube and stored at −80 °C until peripheral blood mononuclear cells preparation.

Biochemical assay

1-Erythrocyte sedimentation rate (ESR in mm/h) was detected by using the Westergren method [17].

2-Serum C-reactive protein (CRP in mg/L) was quantified by using the latex slide semi-quantitative test [18].

3-Rheumatoid factor (RF) was measured by using the slide hemagglutination Rose Waaler test [19].

4-Anti-cyclic citrullinated peptide antibodies (anti-CCP) were measured using commercial ELISA plates coated with second-generation citrullinated peptides [20].

5-Serum osteoprotegerin (OPG) levels were detected using an enzyme linked immunosorbent assay technique (ELISA) [11].

6-Serum anti-CarP levels were detected using an ELISA kit (Cat # MBS7253927, MyBioSource, Inc., San Diego, CA, USA) [21].

7-Receptor activators of nuclear factor Kappa B ligand (RANKL) levels in the serum were determined using an ELISA kit (Cat # MBS2024017, MyBioSource, Inc.) [11].

Bone mineral density (BMD) measurements

The BMD was measured by using DEXA at the following locations: total hip (TH), lumber spine (LS), and the forearm. T-score of −2.5 or less is considered to be diagnostic for osteoporosis [22].

Statistical analysis

Statistical analyses were performed using the SPSS statistical software v21.0. For independent groups, we used the t test. Spearman's test was used to determine the presence of correlation. Multiple logistic regression analyses were used to assess the prediction of osteoporosis by anti-CarP.

Table 1 Demographic data of the two studied groups: Showing significant increase in all laboratory findings and Larsen score and significant decrease in DEXA score in rheumatoid patients as compared with controls by using *t* test

	RA patients (*n*=80)	Controls (*n*=40)
Demographic parameters		
Age, years	46.76 ± 10.03	44.66 ± 7.22
Female, no. (%)	71 (88.8)	34 (85)
Clinical parameters		
Duration of disease (years)	3.5 ±3.4	
DAS28 score	3.64 ± 1.16	
HAQ score	1.83 ± 0.63	
Laboratory parameters (mean ±SD)		
ESR, (mm/h)	45.4 ± 12.4*	10.20±3.49
CRP (mg/dL)	10.9 ±8.3*	3.8±1.85
RF (IU/mL)	90.63 ± 128.61*	6± 1.4
Anti-CCP (units/mL)	98.25 ± 143.98*	10± 4.4
Anti-CarP	135.31±16.48*	30.54 ±11.12
OPG	316± 487*	76±94
RANKL	3.72 ± 0.43*	2.34 ±50.1
DEXA score		
LS	−2.24 ± 0.22*	1.04 ± 0.32
TH	−2.80 ± 8.29*	−1.24 ± 0.22
Forearm	−2.94 ± 9.82*	−1.24 ± 0.22
Modified Larsen score	31.9 ± 11.2	11.8 ± 12.2

*t test is used and significance $P \leq 0.05$
Values are expressed as mean ± standard deviation (SD) or no. and %
DAS28 disease activity for 28 joint indices score, *HAQ* Health Assessment Questionnaire, *ESR* erythrocyte sedimentation rate, *CRP* C-reactive protein, *RF* rheumatoid factor, *Anti-CCP* anti-cyclic citrullinated peptide

Results

Table 1 presents the demographic data from the patients and controls: The mean age of the RA patients was 46.76 ± 10.03 years (range, 30–65 years) and 71 were females (88.8%) and nine were males (11.3%). The control group comprise 40 participants who were matched for age (44.66 ± 7.22years) and gender34 female (85%) and 6 male (15%).

Table 2 presents the number and percentage of positive anti-CarP RA patients according to the DEXA score. The percentage of patients who had osteoporosis in the LS and TH was higher than that for the forearm. Table 3 presents the number and percentage of the patients according to positive laboratory test results.

Anti-CarP was positive in 37.5% of patients. Correlations of anti-CarP with laboratory findings in the patients were shown in Table 4. There was a positive correlation between RF and RANKL and a negative correlation between anti-CCP, ESR, and CRP and OPG.

Correlations between anti-CarP and clinical parameters, DEXA and modified Larsen score in our patients were presented in Table 5, there was positive correlation between anti-CarP and VAS, HAQ, Larsen and DEXA score, and a negative correlation between anti-CarP and DAS score.

Discussion

Carbamylation is a process in which nonenzymatic post-translational modification occurs in lysine residues. Serum anti-CarP antibody levels are increased in a high percentage of RA patients, and the presence of these autoantibodies leads to more severe disease and increased an incidence of bone erosions [23].

Patients with arthralgia who are positive for anti-CarP antibodies show higher prevalence of RA, and these antibodies can be found in patients' serum before symptoms appear [24]. There was a significant increase in anti-CarP positivity in RA patients compared to the normal

Table 2 DEXA score in positive anti-CarP patients: It showed increase percentage of patients who have osteoporosis in LS and TH

	No. (30) (%)
LS ≤ - 2.5	22 (73.33)
> - 2.5	8 (26.66)
TH ≤ - 2.5	20 (66.66)
> - 2.5	10 (33.33)
Forearm ≤ - 2.5	12 (40)
> - 2.5	18 (60)

LS lumbar spine, *TH* total hip

Table 3 Number and percentage of the patients according to laboratory findings positivity: It showed total positive anti-CarP in about 37.5% of the patients

	RA patients no. (%) (*N*= 80)
RF + ve only	11(13.8%)
Anti-CCP +ve only	10 (12.5%)
Anti-CarP +ve only	3 (3.7%)
RF and anti-CCP +ve	25 (31.3%)
RF and anti-CarP +ve	8 (10%)
RF, Anti-CCP and anti-CarP +ve	10 (12,5%)
Anti-CCP and anti-CarP +ve	3 (3.8%)
OPG +ve only	9 (11.3%)
OPG and anti-CarP +ve	3 (3.6%)
RANKL +ve only	7 (8.8%)
RANKL and anti-CarP +ve	3 (3.7%)

control group (Table 1). Anti-CarP positive results were also present in a large percentage of the patients (Table 3). This is in agreement with other study that showed anti-CarP antibodies were elevated in the serum of high percentage of RA patients [25].

We found double positivity in anti-CarP antibodies and anti-CCP in about 3.8% only of patients; there were anti-CarP and RF antibodies in 10% (Table 3). The percentage are slightly more than the results of other study which detect double positivity between anti carp and anti-CCP in 1.1% and between it, and RF is 2.2% only [26] but agree with findings of some studies which detect anti-CarP antibodies in seronegative patients [27]. Presence of many autoantibodies is giving a chance for early and accurate diagnosis of the disease [28].

There was a negative association between anti-CarP and disease activity parameters (ESR, CRP) and anti-CCP (Table 4). This result disagrees with other studies which found that increased disease activity was associated with increased level of anti-CarP in the serum of

Table 4 Correlation between Anti-CarP antibodies and laboratory findings: It showed significant positive correlation with RF and RANKL and negative correlation with other parameters as performed by Spearman's test

	Anti-CarP	
	R	*P*
Anti-CCP	−0.878	<0.001*
RF	0.892	<0.001*
ESR	−0.671	<0.001*
CRP	−0.516	<0.001*
RANKL	0.869	<0.001*
OPG	−0.553	<0.001*

Table 5 Correlation between anti-CarP antibodies with clinical and radiological findings: It showed significant negative correlation with DAS28 and positive correlation with other parameters as performed by multiple logistic regression analysis

	Anti-CarP	
	R	*P*
VAS	0.058	0.221
DAS28	−0.534	<0.001*
HAQ	0.671	<0.001*
Modified Larsen	0.646	<0.001*
T score		
LS	0.287	<0.001*
TH	0.243	<0.001*

LS lumbar spine, *TH* total hip

inflammatory polyarthritis patients [29, 30]. In a recent study, no association was observed between the presence of anti-CarP antibodies and disease activity in rheumatoid arthritis patient [26, 31]. More studies on large number of patients and long-term follow-up are needed to determine the utility of anti-CarP antibodies regarding correlation with disease activity.

There was a positive association between anti-CarP antibodies and RF and HAQ in agreement with Othman et al. who demonstrated that anti-CarP antibodies were linked to increased disease disability in inflammatory polyarthritis patients and increased disability to them [31].

There was a positive correlation between anti-CarP and Larsen score and DEXA score (Table 5) which suggests that anti-CarP may play a role in bone loss, joint erosion, and destruction in RA. This is in agreement with some studies which demonstrated that elevated serum level of anti-CarP antibodies is associated with increased rate of radiographic destruction [26, 31]. Our work also agrees with a study showing decreased bone mineral density in arthritis patients with increased level of anti-CarP antibodies [32], which may be because ACPA and anti-CarP influence osteoclasts activity.

Binding of ACPA to osteoclast cells and their precursors enhances its differentiation and activity and promotes proinflammatory and proosteoclastogenic cytokines release [12]. This is the same mechanism that occurs in positive anti-CarP patients with inflammatory disease.

BMD was decreased more in the LS and TH than in the forearm in patients who were positive for anti-CarP (Table 2), which suggests that the high anti-CarP antibodies titers may lead to systemic bone loss.

In our study, there was positive correlation between anti-CarP and RANKL. There was a negative correlation between anti-CarP and OPG. The OPG/RANKL system is an important regulator of osteoclasts activity [33].

RANKL is expressed by many cells and cytokines and mainly by osteoblasts and synovial cells which is similar to IL1, IL6, and IL17. RANKL is responsible for osteoclasts activation and it is an important factor for bone damage in inflammatory arthritis [34, 35].

Neutrophils, especially neutrophil extracellular tarps (NETs), can lead to the generation of modified autoantigens in RA synovium. Studies have shown that NETs containing carbamylated autoantigens can enhance pathogenic adaptive immunity which leads to production of anti-CarP. Anti-NET protein antibodies can stimulate macrophages to produce proinflammatory cytokines to release RANKL, which promote osteoclast formation and activation. Anti–CarP antibodies can also lead to immune complex formation which can increase osteoclast formation and bone resorption [36].

Previous studies have shown that radiographic progression can be predicted by presence of RANKL or the RANKL/OPG ratio [37, 38]. An increased Larsen score is associated with combined increased RANKL and anti-CarP concentrations [34]. These findings suggest that there is a novel correlation between anti-CarP antibodies and RANKL and radiographic changes in early RA.

Conclusion

In conclusion, anti-CarP antibodies may have an additive diagnostic value and may play a role in the pathogenesis of RA especially in osteoporosis related to RA as it shows positive correlation to RANKL and a negative correlation to OPG. Also, it may be a good predictor of the severity of the disease and play a role in the development of osteoporosis in the rheumatoid patients.

More extensive studies with long-term follow-up are required with more patients to detect its exact role in the disease progression and osteoporosis.

The limitations of our study are the relatively small number of patients; also, we did not perform ultrasonographic study for our patients which could detect the erosions earlier that X-ray. Future studies are recommended with long-term follow-up to highlight the relationship between the anti-CarP and osteoporosis.

Abbreviations

anti-CarP: Anti-carbamylated protein; RA: Rheumatoid arthritis; RF: Rheumatoid factor; Anti-CCP: Anti-cyclic citrullinated peptide; DAS28: Disease activity for 28 joint indices score; HAQ: Health Assessment Questionnaire; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; OPG: Osteoprotegerin; RANKL: Receptor activator of nuclear factor κB ligand; BMD: Bone mineral density; LS: Lumbar spine; TH: Total hip

Acknowledgements

Not applicable.

Authors' contributions

MH and ER authors had contributed to the conception, design of the work, the acquisition, analysis, interpretation of data, had drafted the work and substantively revised it, and finally, had agreed both to be personally accountable for their own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which they were not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature. HE contributed to collecting the patient's data (history, clinical and investigation data), processed it in the patient excel sheet, and also contributed in statistical analysis and writing the manuscript. RG is a major contributor in methodology, analyzed and interpreted the patient's data regarding the objectives and methods of research supervision of the steps of writing and results reviewing together with the final manuscript. All authors had read and approved the final manuscript.

Funding

The study has no funding from any source.

Availability of data and materials

Not applicable

Declarations

Ethics approval and consent to participate

Written informed consents were obtained from all the participants before entering the study.
The study was approved by the research ethics committee of the Tanta University, Faculty of Medicine (approval code (34206-10-19)).

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Received: 23 October 2020 Accepted: 26 February 2021
Published online: 23 March 2021

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