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Evaluation the Serum Level of the Carbamylated Protein Antibody Compare with the Anti-Citrullinated Peptide Antibody and Rheumatoid Factor Antibody in Rheumatoid Arthritis Patients

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ABSTRACT

Background: Rheumatoid arthritis is an autoimmune disease that causes irreversible joint destruction due to persistent synovitis. ACPA and RF are gold standard antibodies for diagnosis, many studies shown that Anti-CarP can be utilized with ACPA.

Objective: Comparison of Anti-CarP with ACPA and RF serum levels in RA patients based on disease severity.

Materials and Methods: Case-control study includes 270 subjects from October 2022 to April 2023 in Al-Mujtaba Hospital/Rheumatology Unit, separated into 180 RA patients and 90 controls, then into 90 seropositive and 90 seronegative RA cases. All patients consented to ACR 2010 rheumatologists' diagnoses; venous blood samples were collected and split into sodium citrate tubes for Westergren ESR and gel tubes for serological assays, C-RP and RF employing Nephelometry, ACPA, and anti-CarP by ELISA. SPSS 26 was used for statistical analysis.

Results: RA cases were 180, divided into 90 seropositive and 90 seronegative RA patients, mean of Anti-CarP highly significant in seronegative than seropositive at P.value (0.000), in mild and moderate DAS28-CRP at P.value (0.001), and significant in regulars treatment than irregulars at P.value (0.047), while ACPA and RF highly significant in seropositive than seronegative at P.value (0.000), in severe DAS28-CRP at P.value less than (0.01), and in irregulars treatment than regulars at P.value (0.001).

Conclusions: Anti-CarP Ab associated with RA patients especially seronegative RA so may be useful as diagnostic marker complementary with RF and ACPA, associated with mild and moderate DAS28-CRP and regulars treatment compare to the ACPA and RF so could be used as prognostic marker for disease severity.

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Introduction

Rheumatoid arthritis (RA) is a disease of unknown origin that is characterized by inflammatory alterations in synovial tissue, cartilage, and bone, as well as, less commonly, extra-articular locations (1), the morning stiffness of the afflicted joints for more than 30 minutes, exhaustion, fever, weight loss, sensitive, swollen, and heated joints, and rheumatoid nodules under the skin are common symptoms of RA (2).

Rheumatoid arthritis is the most prevalent, affecting 0.5 to 1% of the world's population, with a 3-fold higher incidence in women than in men (3), although there is no known cause of RA, there are several risk factors that are linked to a higher chance of having the illness, include smoking, poor oral health, viral infections, and a family history of RA or other autoimmune disorders(4).

The clinical picture of RA is the consequence of a tight interaction between cells, soluble mediators, autoantibodies, and innate and adaptive immunological signal transduction pathways, all of which are implicated at different phases of the illness (5), multiple antibodies have been identified in rheumatoid arthritis based on the antigens to which these antibodies attach. Anti-citrullinated peptide antibodies (ACPAs) and rheumatoid factor (RF) are current diagnostic serum biomarkers included in the 2010 American College of Rheumatology (ACR)/European League of Rheumatism (EULAR) RA classification criteria (6).

Antibodies against carbamylated protein antigen (anti-CarP antibodies) were discovered in patients with RA in 2011, and subsequent research has established the predictive and prognostic value of this antibody system (7).

Anti-citrullinated peptide antibody is significantly more specific for RA than RF (95% for ACPA versus 85% for RF). Anti-CarP is most prevalent in ACPA-positive RA patients but is also present in 8%–14% of ACPA-negative patients (8). The anti-CarP antibody is useful biomarker for diagnosing RA, particularly in ACPA-negative patients (9).

Anti-Citrullinated Peptide Antibody is associated with more severe structural injury, radiographic progression, and an inferior therapeutic response(10). There was a correlation between the presence of RF and ACPA autoantibodies and worsening disease symptoms, joint injury, and increased mortality (11).The anti-CarP antibody may improve the likelihood of tailoring the initial treatment to the patient, it may also be possible to add anti-CarP to the panel of markers used to establish a pre-RA diagnosis(12).

In the subgroup of anti-CarP-positive patients, there was a significant reduction in anti-CarP titer and disease activity score28-C-reactive protein (DAS-28 CRP) after treatment, therefore, anti-CarP could be used as a good clinical response predictor(13).

The purpose of the investigation was to compare the serum levels of ACPA, RF, and anti-CarP in all patients as a disease activity related prognostic factor.

Subjects and Method

Case-control study, at Imam Al-Hassan Al-Mujtaba Hospital/Rheumatology Unit patients was included. From October 2022 to April 2023, 270 participants were divided into two main groups [180 RA patients and 90 healthy control subjects], and then the RA cases were further divided into two subgroups [90 seropositive and 90 seronegative cases]. The RA patients were 180 (20 males and 160 females), the seropositive cases were 90 (11 males and 79 females) and the seronegative cases were 90 (9 males and 81 females). All patients were diagnosed by rheumatologists in the rheumatology department of Imam Al-Hassan Al-Mujtaba Hospital in Karbala. Ethically, the permission had been taken from the college of medicine at Karbala University and the Iraqi Ministry of Health/Karbala Health Directory, Imam Al-Hassan Al-Mujtaba Hospital/Rheumatology Department, and from the patients about collecting blood

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samples and using their data for research purposes; all of them had six criteria or more from the 2010 ACR/EULAR, and those patients with RA disease did not have other rheumatological disorders or other autoimmune disorders or infectious diseases.

Patients and controls had five milliliters of venous blood extracted for estimation of Erythrocyte Sedimentation Rate ESR, CRP, RF ACPA, and Anti-CarP Ab using the western green method for ESR-kits, AFCCO/Jordan. 1.6 ml was added to the ESR tube, which contained 0.4 ml of sodium citrate anticoagulant. The remaining volume was added to the gel tube and centrifuged to separate the serum, which was then preserved at -20^{o} in four small Eppendorf tubes for serological analysis. CRP -titer kits, Hipro Biotechnology/China and RF test-titer kits by Nephelometry, Hipro Biotechnology/China, ACPA in-direct-ELISA kits, Sun Long Biotech/China and Anti-CarP in-direct-ELISA kits, China's Sun Long Biotech, Calculator for the disease activity score (DAS) (the severity of the disease) mild, moderate and severe, The statistical analysis was performed using version 26 of SPSS.

Results

1. Distribution of the Sex, Age and Age groups

The study included 270 subjects, the total RA cases were 180 patients females 160 (88.9%) and males 20 (11.1%), with the mean Standard Error (SE) of age 49.82 (0.86) and the age range (20-73), the seropositive RA cases were 90 patients females 79 (87.8%) and males 11 (12.22%) with the mean (SE) of age 47.73 (1.208) and the age range 20–73 years, the seronegative RA cases were 90 patients females 81 (90%) and males 9 (10%) with the mean (SE) of age 51.90 (1.195) and the age range 21–73 years, the highest frequencies of females sex than males show in both seropositive and seronegative RA patients, the healthy control group were 90 subjects females 78(86.7%) and males 12(13.3%) with the mean (SE) of age 49.00 (0.991) and the age range 21–70 years. According to the age variable in the present investigation, the patients are divided into three groups: Group one (20–39 years) has 33 (18.3%) members, Group two (40–59 years) has 113 (62.8%) members, and Group three (60–79 years) has 34 (18.9%) members. Group two has the maximum percentage.

2. Comparison between RA patients and Healthy Control Group

Regarding the comparison between RA patients and health controls, the variables of the present investigation, Disease Duration (D. Duration), ESR-level, CRP-level, RF-titer, ACPA and Anti-Carp, show highly statistically significant for the RA patients group compare to the healthy control group at *P.*value (0.000) but the mean (SE) of the age variables show no statistically significant differences between RA patients group and healthy control group, as shown in the Table 1.

Table 1: Comparison between RA patients and Healthy Control Group

Parameters	**RA Patients N=180**	**Control N=90**	**Sig**
	mean(SE)	**mean(SE)**	
Age	49.82 (0.86)	49.0 (0.99)	0.508^{NS}
D. Duration	6.30 (0.43) *	0.00 (0.00)	0.000**
ESR-level	38.73 (1.64) *	6.83 (0.21)	0.000**
C-RP-titer	18.61 (2.06) *	0.95 (0.037)	0.000**
RF-titer	43.55 (4.29) *	5.44 (0.23)	0.000**
ACPA	55.81 (4.24) *	3.97 (0.096)	0.000**
Anti-CarP	4.43 (0.49) *	2.29 (0.21)	0.000**

NS: Non-Significant **: Highly significant *P.* value < 0.01

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3. Comparison between Seropositive and Seronegative RA patients

The comparisons between the two RA patients groups (seropositive and seronegative) according to the current study variables show no statistically significant in the mean (SE) of the age, disease duration, ESR-level and CRP-titer between seropositive and seronegative RA patients while there is highly statistically significant differences show in the mean (SE) of the RF-titer and ACPA for seropositive than seronegative RA patients at *P.*value (0.000), but the mean (SE) of Anti-CarP show highly statistically significant differences for seronegative than seropositive RA patients at *P.*value (0.000), as shown in the Table 2.

Table 2: Comparison between Seropositive and Seronegative RA patients

Parameters	Seropositive mean(SE)	Seronegative mean(SE)	P. Value
Age	47.73 (1)	51.9 (1)	0.182^{NS}
D. Duration	5.94 (0.6)	6.69 (0.6)	0.173 ^{NS}
ESR-level	36.66 (2)	40.81 (2)	0.809 ^{NS}
C-RP-titer	17.8 (2.62)	19.42 (3.2)	0.986 ^{NS}
RF-titer	75.53(7.08)*	11.56 (1.1)	0.000**
ACPA	94.4 (6.22) *	17.21(0.43)	0.000**
Anti-CarP	4.17 (0.92)	4.68(0.36)*	0.000**

NS: Non-Significant **: Highly significant *P.* value < 0.01

4. Comparison of Study Variables Regarding to DAS28-CRP

The mean (SE) of the age variables show no statistically significant among the three activity groups (mild, moderate and severe), while the mean (SE) of the disease duration, ESR-level, CRP-titer, RF-titer and ACPA show statistically significant differences at *P.*value less than (0.01) for severe activity group than mild and moderate, but the mean (SE) of the Anti-CarP show highly statistically significant differences at *P.*value (0.001) for mild and moderate than severe activity group, as shown in the Table 3.

Table 3: Comparison of Study Variables Regarding to DAS28-CRP

Parameters	Mild mean(SE)	Moderate mean(SE)	Severe mean(SE)	Sig
Age	47 (2)	51 (1)	51 (2)	0.180 ^{NS}
D. Duration	4.2 (0.5)	6.4 (0.5)	8.2 (1.2) *	0.001**
ESR-level	27 (2)	35 (2)	59 (4) *	0.001**
C-RP-titer	6.26 (1.10)	11.39 (1.08)	48.1(6.87)*	0.001**
RF-titer	34.91 (5.87)	33.45 (4.46)	76.35(13.3)*	0.001**
ACPA	62 (9.48)	45.10 (4.82)	75.63(10.6)*	0.004**
Anti-CarP	5.25 (1.99) *	4.37 (0.4) *	3.76 (0.25)	0.001**

NS: Non-Significant *Significant *P.* value < 0.05 **highly significant *P.* value <0.01

5. Comparison of Study Variables Regarding to Treatment Regularity

The mean (SE) of the age variable show no statistically significant between regulars and irregulars treatment intake among RA patients, while the mean (SE) of disease duration, ESR-level, CRP-titer, RF-titer and ACPA show highly statistically significant differences at *P.*value (0.001) for irregulars treatment intake than regulars, but the mean (SE) of Anti-CarP show statistically significant differences at *P.*value (0.04) for regulars treatment intake than irregulars, as shown in the Table 4

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Table 4: Comparison of Study Variables Regarding to Treatment Regularity

Treatment Status	Regulars mean (SE)	Irregulars mean (SE)	P. Value
Age	50 (1)	50(1)	0.902 NS
D. Duration	5.4 (0.5)	7.9 (0.8) *	0.001**
ESR-level	32 (2)	50 (3) *	0.001**
C-RP-titer	12.12 (1.55)	29.57 (4.62) *	0.001**
RF-titer	31.46 (3.61)	63.93 (9.35) *	0.001**
ACPA	51.21 (4.83)	63.56 (7.92) *	0.001**
Anti-CarP	4.72 (0.75) *	3.93 (0.42)	0.047*

NS: Non-Significant *Significant *P.* value < 0.05 **highly significant *P.* value <0.01

Discussion

1. Distribution of the Sex, Age and Age groups

The present study involved of 270 participants, who were divided into two groups (case-control). The mean age of the participants in the case and control groups was 49.8 and 49.0, respectively. The age range of the participants varied from 20 to 73 years old. Furthermore, within the RA cases, participants were further classified based on serological tests into seropositive individuals with a mean age of 47.73 and seronegative individuals with a mean age of 51.9. The findings of the study indicated that there was sufficient similarity in terms of mean age and age range among the different study groups. These results are consistent with previous studies conducted by Abbood, 2019 in Iraq (14), which reported mean ages of 48.46 for RA cases and 48.85 for controls, Hussein, 2019 in Iraq (15), which found a mean age of 47.82 for RA patients and 46.82 for controls, and Kolarz et al.,2021 in Poland (12), which reported a mean age of 52.1 and an age range of 18–70.

In terms of age groups in the current study, group two has the highest numbers, with 113 subjects representing 62.8% of the age group from 40 to 59 years. These frequencies are in agreement with Abbood's, 2019 findings from Iraq (14), which showed that patients' ages ranged from 40 to 59 years, with 112 subjects representing 62.22% of the study group, and in disagreement with Deraj's, 2021 findings from Iraq (16), which showed that patients' ages ranged from 40 to 59 years, with 46% representing the age group. This discrepancy may have resulted from the study's different inclusion and exclusion criteria used in the selection of RA patients.

Regarding the sex distribution in the study, it appears that females constitute the majority of RA patients, both in seropositive and seronegative cases (87.8%–90%) of the sex factor among the present study groups. This result is in agreement with the findings of Favalli et al., 2019 in the United States (17), which showed that females occur more frequently than males in a variety of different studies. It is also in agreement with the findings of Iannone, 2017 in Italy (18), that showed the female 77 (80.68%) of total RA patients in their study. While the study disagrees with Jawaheer et al., 2012 in North America (19), that showed females in early RA were 2263 (75%) of the total 3017 patients with RA, also disagree with Nilsson et al., 2021 in Sweden (20), that showed the female sex represented 68% of RA patients. This disagreement may be due to the greatest sample size obtained in this study as a cohort study design.

2. Comparison between RA patients and Healthy Control Group

As shown in Table1, there are no statistically significant differences in age between the RA patients and control group at *P.*value 0.5. These results are consistent with Sveinsson et al., 2020 in Sweden (21) , which suggests that in case-control studies, age and sex should be matched or semi-matched, and with Hussein, 2019 in Iraq (15), which found no statistically significant differences at *P.*value (0.58) between patient and control.

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These results are consistent with Hussein, (15), Abbood, (14), Flyeh, (22) in 2019, and Deraj, 2021 (16) in Iraq, regarding disease duration, ESR, CRP, RF, and ACPA statistically significant differences with *P*.values less than (0.01) for RA patients than healthy control group . All of these studies demonstrated that all of the aforementioned variables had significant at *P*.value less than (0.01).

The anti-CarP were statistically significant at *P*.value less than 0.01 for RA patients than the healthy control group. This result is consistent with Elsayed et al., 2019 (23), Mohammed et al., 2020 (24) in Egypt. Anti-CarP levels varied significantly between RA patients and healthy controls in each of these studies.

3. Comparison between Seropositive and Seronegative RA patients

Concerning the results shown in Table 2, that show the age, disease duration, ESR, and CRP are not statistically different between seropositive and seronegative RA, this result agrees with Kotecki et al., 2021 in Poland (25), this study showed that there is no significant difference based on age, disease duration, ESR, and CRP at *P.* value less than (0.05) among RA patients, also agreeing with Oweis et al., 2020 in Jordan (26) that demonstrated no statistically significant difference in the mean of ESR among RA patients at *P.* value (0.96).

The results in Table 2, also show that the RF and ACPA are statistically significant at *P*.value (0.000) for seropositive RA patients compared to seronegative RA patients, and it is rationally accepted that RA patients are classified according to these markers, so that these results agree with Kronzer et al., 2021, in Sweden (27), which demonstrated that the ACPA and RF were specific for seropositive RA patients, and Reed et al., 2020, in Sweden (28), which demonstrated that RA patients were classified as seropositive or seronegative, depending on the presence or absence of ACPA and RF.

Anti-CarP results are statistically significant at *P*.value (0.000) for seronegative RA patients compare to seropositive RA patients. The anti-CarP antibody results agree with Sidiras et al., 2021 in Belgium (29), which said that anti-CarP antibodies were linked to seronegative RA. They also agree with Lamacchia et al., 2021 in Switzerland (30), which found a significant difference for seronegative patients compared to seropositive patients at *P*.value (0.02), and with Wang et al., 2023 in China (31), which said that anti-CarP antibodies may help diagnose RA early.

4. Comparison of Study Variables Regarding to DAS28-CRP

According to the findings in Table 3, the results were compared with the mean of the study variables on the basis of DAS28-CRP, so the result of mean age among mild, moderate, and severe groups is non-significantly different, while the acute phase reactants (ESR and CRP) are statistically significant at *P*.value less than (0.01) among the DAS28-CRP groups, particularly for severe rather than moderate and mild groups, and these findings agree with Hussein, 2019 in Iraq (15) that, showed a statistically significant difference for severe DAS28-CRP in RA patients compared to the other two groups and with Abbood, 2019 in Iraq (14), that demonstrated a highly significant value for the mean of ESR and CRP for severe groups compared to mild and moderate DAS28-CRP.

The ACPA and RF are likewise related to a higher DAS28-CRP group than the other groups, with a highly significant *P.* value less than (0.01), which agrees with the findings of the following research Abbood, 2019 (14), Flyeh, 2019 (22), Hussein, 2019 (15), and Deraj, 2021 (16) in Iraq . All of these studies found that ACPA and RF were positively linked with disease activity, particularly in severe patients.

The anti-CarP antibodies are related to the mild and moderate groups, as shown clearly in Table 3. These findings are consistent with Wang et al., 2023 in China (31), who revealed that anti-

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CarP Abs are connected with mild DAS28 and may be used as a novel marker in conjunction with other markers.

5. Comparison of Study Variables Regarding to Treatment

Regularity

The results of the Table 4, which compares the study variables between the regular and irregular groups according treatment intake, indicate that the irregular group had significantly longer disease duration. The irregular group's ESR, CRP, RF, and ACPA were significantly higher than those of the regular group. These findings are consistent with the findings of the following studies: Abbood, 2019 (14) and Hussein, 2019 (15) in Iraq, who both documented the significant mean differences for patients receiving irregular treatment regarding the ESR, CRP, RF, and ACPA level being high in the irregular group, and Ghaseminasab-Parizi, 2022 in Iran (32), who demonstrated that the regular specific treatment received by RA patients was significantly lower disease activity score.

The results of the anti-CarP Abs in Table 4 show significant differences with the regular group of RA patients at P. value less than (0.05) These results agree with Zeng et al., 2020 study in China (33), This study documented the high level of anti-CarP Abs with regular treatment intake and can be used as prognostic marker for RA patient follow-up.

Conclusion

Anti-CarP antibodies can be helpful in the diagnosis of RA patients, especially seronegative RA, and can be used in combination with the gold standard antibodies ACPA and RF, Anti-CarP also associated with mild to moderate disease activity and regular treatment of RA patients, so can be used as a prognostic marker when compared with ACPA and RF, which are associated with severe disease activity and irregular treatment of RA patients.

Recommendation

- Additional research on other antibodies in seronegative RA patients.
- Additional studies with a bigger sample size are needed to assess the serum levels of anti-CarP in RA patients.
- More research is needed to determine the possible importance of anti-CarP in RA etiology in order to clarify their utility in disease activity, treatment monitoring, and disease progression, particularly in seronegative RA patients.

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