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DIAGNOSTIC VALUE OF SERUM ANTI-CARBAMYLATED PROTEIN ANTIBODIES IN IRAQI PATIENTS WITH RHEUMATOID ARTHRITIS: A CASE CONTROL STUDY

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ABSTRACT
Rheumatoid arthritis (RA) is a progressive inflammatory disorder characterized by proliferation of the synovial membrane and persistent uncontrolled inflammation resulting in chronic destructive polyarthritis. To evaluate the serum level of anti-carbamylated protein antibodies in RA and to demonstrate its relation with baseline characteristics of the patients, the study included 50 Iraqi rheumatoid arthritis patients and 35 controls. Age, body mass index, gender, and smoking status were recorded Rheumatoid factor (RF), anti – cyclic citrullinated peptide (ACPA), highly sensitive C – reactive protein (hs – CRP), and anticarbamylated protein were measured in both groups. Age, BMI were significantly higher in RA patients compared to controls, the female to male ratio was 5.25:1 in RA, and 1.7:1 in controls. Serum levels of ACPA and RF were significantly higher in RA patients compared to controls. Serum Anti- carbamylated protein and hsCRP were higher in RA patients compared to controls ($p>0.05$). RF had an excellent ability to discriminate between RA and controls), the rest of the variables (ACPA, CRP, and anticarbamylated protein) had poor ability to discriminate between RA and control. At optimum cut off value >2.735 Anticarbamylated protein has highest accuracy 67.0% and very high specificity 97.1% and PPV 95.8% but low sensitivity 46% and NPV 55.7%. serum anticarbamylated protein antibody was significantly higher in RA patients compared to controls but it was not a valid measure to differentiate between patients and controls. No significant effect of baseline characteristics on anticarbamylated protein antibody.

KEY WORDS: anticarbamylated protein antibody, RA, immunological markers.

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by inflammatory polyarthritis, which affects peripheral joints, especially the small joints of the hands and feet. Chronic untreated inflammation may lead to joint erosions and joint destruction. Rheumatoid arthritis affects about 0.5% to 1% of the population world-wide[1]. RA is associated with substantial morbidity and accelerated mortality and exerts a tremendous economic toll on affected patients, their families, and society[2]. The pathogenesis of RA is largely unknown but heritability of approximately 50% for ACPA-positive and approximately 20% for ACPA-negative RA has been shown, and environmental factors are believed to interplay with genetic factors during the early, pre-symptomatic stages[3]. Smoking and periodontal disease have been linked to development of mainly seropositive RA[4]. Obesity could be another possible environmental risk factor due to the pro-inflammatory nature of the adipose tissue, but studies evaluating the association between body mass index (BMI) and the risk of RA have shown contradictory results[5]. C-reactive protein (CRP) is an acute-phase reactant that increases within 4 to 6hours of inflammation or acute tissue injury. It can be tested by different assays with different measuring ranges. Previously, lower measuring limits of 3 to 10mg/L were common, whereas during recent years, analytical methods optimized to measure results as low as 0.1mg/L have become widely used, often called high-sensitivity CRP (hs-CRP) methods[6]. Serum RF is closely associated with RA and may help confirm a suspected diagnosis of the disease. Also RF is an autoantibody (usually to IgM) that binds to the FC part of IgG and is present in 75% to 85% of patients meeting the ACR criteria for RA will be seronegative for RF [7].

Importantly RF may be absent in the first few months of RA and may become positive with persistence of synovitis. Thus RF may only be found in 40% to 50% of patients with ≤ 5 months of symptoms[8]. ACPAs, being detected in 60–80% of RA sera, are more specific for RA, as they are rare in other diseases and only present in approximately 2% of the healthy population [9]. ACPAs as well as RFs, have been detected in the serum of RA patients years before the onset of RA [10], suggesting that the development of RA occurs long before the appearance of symptoms.

Humoral responses to homocitrullinated proteins (subsequently referred to as ACarP antibodies; ACarP Ab) have been reported in both patients with early and established seropositive RA, as well as by a proportion of seronegative RA patients. Indeed, ACarP Ab, like ACPA, can be found in patient sera years before the onset of RA, with a median time of approximately five years from first serologic appearance to the onset of clinical signs and symptoms [11].

PATIENTS & METHODS

The study included 50 Iraqi RA patients fulfilled 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) Classification Criteria for diagnosis of RA [12]. These RA patients were form Rheumatology consultation clinic /Baghdad Teaching Hospital during the period from March to July 2017. The patients compared with another 35 healthy controls. Patients were excluded from the study if they had another overlapping inflammatory arthritis or connective tissue disease that can affect serum level anticarbamylated protein antibody.

Full history was taken and complete clinical examination was done for each participant in the study and nd laboratory features as RF, ACPA, CRP, and anticarb-amylated protein antibody were measured.

Eight to ten ml of venous blood had drawn from each individual of the two groups under complete aseptic condition. The blood samples were collected in anticoagulant – free tubes used for separation of serum to detect RF, CRP, ACPA and anti-carb. Two ml was collected in ESR tube. Immunological assays were worked out in the medical laboratories of Nursing home hospital. Quantitative measurement of rheumatoid factor (RF), screen rheumatoid factor, anti – cyclic citrullinated peptide (ACPA), highly sensitive C – reactive protein (hs – CRP), and anti Carbamylated protein antibody

RESULTS

The mean Age and BMI were significantly higher in RA patients compared to control, the percentage of female was significantly higher in RA compared to control 845vs 62.86%, p=0.026) as shown in table1.

TABLE 1: demographic, clinical, and Lab features of patients and controls

	Control	RA	P value
Number	35	50	-
Age (years)	27.54 ± 4.54	47.62 ± 10.43	<0.001
BMI	26.47 ± 3.61	29.86 ± 5.44	0.001
Gender, no.(%)			0.026
Female	22 (62.86%)	42 (84.00%)	
Male	13 (37.14%)	8 (16.00%)	
Smoking, no.(%)			0.354
Non-smoker	30 (85.70%)	46 (92.00%)	
Smoker	5 (14.30%)	4 (8.00%)	

The median (interquartile range) serum anticarbamylated protein antibody was obviously higher in RA patients compared to controls (2.32 (1.61 – 6.07) vs 1.99 (1.79 – 2.54), p=0.282) as in figure 1.

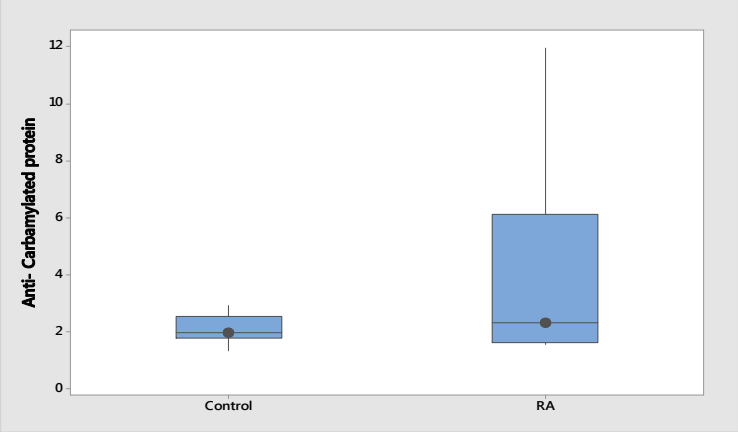

FIGURE 1: boxplot of Anti- carbamylated protein in RA and control

Table (2) shows the comparison of serum levels of different immunological markers (anti-carb, CRP, ACPA and RF) between patients and control groups.

Serum levels of ACPA and RF was significantly higher in RA patients compared to control, anti-Carbamylated protein and CRP had higher serum levels in RA patients however it was not statistically significant, as illustrated in table 2.

TABLE 2: Serum levels of different immunological markers between patients and controls

	Control	RA	P value
Number	35	50	-
Anti- Carbamylated protein	1.99 (1.79 – 2.54)	2.32 (1.61 – 6.07)	0.282
CRP	7.09 ± 2.15	7.81 ± 1.59	0.097
ACPA	1.79 (1.47 – 2.50)	2.32 (1.64 – 3.98)	0.024
RF	1.9 (4.9 – 12.1)	104.2 (25.9 – 485)	<0.001

RA, Rhuematoid factor; CRP, C - reactive protein; ACPA, anticitrolinated protent antibody; RF, rheumatoid factor. RF had an excellent ability to discriminate between RA and controls, the rest of the variables had poor ability to discriminate between RA and controls as shown in Table 3.

TABLE 3: ROC curve for different immunological markers between to Dxx patients and controls

Variable	AUC	95%CI AUC	P value
Anti- Carbamylated protein	0.569	0.457 to 0.676	0.287
ACPA	0.644	0.533 to 0.745	0.018
CRP	0.533	0.421 to 0.642	0.664
RF	0.955	0.887 to 0.988	<0.001

CRP, c-reactive protein; ACPA, anticitrolinated protent antibody; RF, rheumatoid factor; AUC, area under the curve; ROC, receiver operating characteristics. The validity of anti-carbamylated protein antibody, ACPA, CRP and RF are shown in table 4. At optimum cut off value >2.735 Anticarbamylated protein has highest accuracy 67.0% and very high specificity 97.1% and PPV 95.8% but low sensitivity 46% and NPV 55.7%.

TABLE 4: validity parameters for different immunological markers

Variable	Cut point	Sensitivity	Specificity	Accuracy	PPV	NPV
Anti- Carbamylated protein	>2.735	46%	97.1%	67.0%	95.8%	55.7%
ACPA	>2.05	66%	65.7%	66.4%	73.3%	57.5%
CRP	≤8.53	92%	42.9%	67.5%	56.4%	39.1%
RF	>18.398	86%	100%	93.0%	100%	83.3%

CRP, C - reactive protein; ACPA, anticitrolinated protent antibody; RF, rheumatoid factor

There was no significant correlation between demographic features, clinical features, and laboratory investigations with anticarbamylated protein antibody as shown in table 5.

TABLE 5: Correlation between demographic features, clinical features, and Lab with anti Carbamylated protein

Variables	Anti- Carbamylated protein	
	Beta	P value
Age	0.190	0.187
BMI	-0.121	0.403
Disease Duration	0.044	0.763
Number of tender joints	0.115	0.428
Number of swollen joints	0.209	0.145
Patient Global assessment	0.083	0.568
Evaluator global assessment	0.113	0.433
CDAI	0.168	0.243
SDAI	0.187	0.192
DAS-28ESR	0.132	0.362
ESR	0.052	0.718
ACPA	-0.099	0.493
RF	0.191	0.185
CRP	0.170	0.239

BMI, body mass index; CDAI, clinical disease activity index, SDAI, simplified disease activity index, DAS-28 . disease activity score 28; ESR, erythrocyte sedimentation rate; CRP, c-reactive protein; ACPA, anticitrolinated protent antibody; RF, rheumatoid factor.

DISCUSSION

Rheumatoid arthritis (RA) is a progressive inflammatory disorder characterized by proliferation of the synovial membrane and persistent uncontrolled inflammation resulting in chronic destruction polyarthritis. Typically, RA manifests as a symmetric arthritis involving numerous small and large joints[13]. Rheumatoid factor (RF) is present in more than 80% of patients with RA, and may be detectable in the synovium (but not the blood) of some seronegative RA patients. Low affinity physiological RF is produced transiently in infectious disease, in which it may facilitate clearance of immune complexes by causing aggregation into larger complexes [14]. In recent years the treatment of rheumatoid arthritis advanced and as soon as possible active drugs should be given to the patients [15]. In the current study we aimed to evaluate the levels of anticarbamylated protein in RA and to demonstrate their relation with other immunological markers of rheumatoid arthritis and disease activity. This study showed that serum levels of ACPA and RF were significantly higher in RA patients compared to controls. Also, serum ant-carbamylated protein antibody and CRP were higher in RA patients however it was not statistically significant. Also the study showed that RF had an excellent ability to discriminate between RA and controls. Antibodies to carbamylated proteins antibody have been detected in the serum of 36-45% of RA patients. Anti-carp antibodies have been shown to be associated with the development of

RA in patients with arthralgia and more severe radiological progression in the total and ACPA-negative RA population [16].

A non-significant increase of serum anti-carbamylated protein antibodies was found in the present study when compared with healthy controls In contrast to other studies which reported significant increase of anti-carp antibodies in RA patients[17-19] possible explanation is the small sample size in our study.

In this study we observed that RF as the main serum marker to use to diagnose RA. A recent study has reported that anti-carbamylated protein antibodies were found to be significantly elevated in RA patients and significantly associated with health assessment question (HAQ) which may provide evidence that anti-carbamylated protein antibodies could be potential biomarker for diagnosis of RA in patients [20]. In a study in large cohort of Italian patients. Anti-carbamylated protein antibodies demonstrated relatively low sensitivity and slightly higher specificity compared to ACPA and RF. Anti-carbamylated protein antibodies were detected in variable percentages of patients with other autoimmune rheumatic disease and their generation could be attributed to the inflammatory status [21].

CONCLUSION

Serum anticarbamylated protein antibody was significantly higher in RA patients compared to controls but it was not a valid measure to differentiate between patients and controls. No significant effect of baseline characteristics on anticarbamylated protein antibody.

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