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Research Paper

Anti-Carbamylated Protein Antibodies in a Sample of Iraqi Patients with Systemic Lupus Erythematosus

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ABSTRACT:

BACKGROUND:

Systemic lupus erythematosus is an autoimmune illness characterized by extensive immune complex deposition and consequent tissue damage. It is distinguished by the presence of antinuclear antibodies and the involvement of several organ systems.

OBJECTIVE:

Is to assess serum anticarbamylated protein antibody (anti-CarP) level in SLE patients and evaluate the association between anti-CarP antibodies with different disease characteristics.

SUBJECTS AND METHODS:

Serum level of anti-CarP antibody was measured for a total of 40 adult female patients with SLE who attended the Rheumatology Consultation Clinic of Baghdad Teaching Hospital / Medical City, compared with 40 apparently healthy controls matched in ages and sexes to the patients using Enzyme Linked Immuno-Sorbent Assay (ELISA) technique which is also used to measure serum anti ds-DNA levels.

RESULTS:

Serological data showed that the serum level of anti-CarP antibody was significantly higher in the SLE patients compared with controls p value (0.001). In SLE patients there was a significant correlation between anti-CarP antibody and both body mass index and serum ds-DNA antibody levels, however, did not correlate with SLEDAI score.

CONCLUSION:

Serum anti-Carp antibody level was able to differentiate between patient with SLE and healthy control with sensitivity 80%, specificity 77.50% and accuracy 78%.

KEYWORDS: Systemic lupus erythematosus, anticarbamylated protein antibody, Enzyme Linked Immuno-Sorbent Assay, double stranded-DNA, Systemic lupus erythematosus disease activity index.

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INTRODUCTION:

Systemic Lupus Erythematosus (SLE), is a chronic autoimmune illness. Several organs involvement is a distinguishing feature. The precise cause of lupus is unknown, although it involves a complex combination of immune system dysregulation, genetic susceptibility, and a number of environmental variables[(1)].

One recently identified type of autoantibodies is anticarbamylated protein (anti-CarP) antibody. Isocyanic acid covalently binds to proteins to cause a nonenzymatic reaction called carbamylation. The creation of covalently bound adducts in proteins known as post-translational alterations-derived products, which are in charge of altering protein structure and functional characteristics, sets these post-translational alterations apart. Neoepitopes produced by protein post-translational alteration have the power to trigger immune system reactions. One of the main mechanisms that initiates an inflammatory response may be the carbamylation of lysine residues to generate homocitrulline[(2)].

SUBJECTS AND METHODS:

From January 2022 to September 2022, this case control research was conducted at the Baghdad Teaching Hospital's Rheumatology Consultation Clinic in Medical City. The Department of Medicine at the University of Baghdad provided ethical approval. Before beginning to participate in this study, each subject gave their informed permission. A total of 40 systemic lupus erythematosus (female patients) were involved in this study. They were on regular treatment. All the patients in the study fulfil the American College of Rheumatology (ACR) 1997 SLE diagnostic criteria. Disease activity was evaluated according to the disease activity index for lupus patients (SLEDAI) and the patients divided into 4 groups (mild, moderate, high and very high). Patients under the age of 16 and patients with overlap syndromes were excluded.

Blood sample collection and preparation

Each participant had five milliliters of blood drawn using a septic method. Following the evacuation of the blood samples into gel tubes and centrifugation to extract serum, the supernatant was carefully transferred to Eppendorf tubes and stored in aliquots at -20°C until the anti-CarP antibody ELISA test was conducted.

Human anti-carbamylated protein Antibody (ACP-Ab) Enzyme-linked Immunosorbent Assay (ELISA) ACP-Ab serum level was measured in accordance to the manufacturer's instructions with the use of ACP-Ab ELISA kit (Biont / China).

Analysis of statistics

The collected data was introduced into Social Science Statistical program (IBM SPSS statistics Version 26). Chi square test was used to find out significances' association between related categorical data. The correlation test was used to find out significance of correlation between related variables. ROC curve was used to find out significance of anti-CarP antibody as a screening test to detect SLE. P value(P) less than 0.05 was considered as a discrimination point for significance.

RESULTS:

In the current study the mean age ± SD of (SLE) group was (34.8±11.3 year) which is not significantly differ than that of control group (33.3±7.4 year). P (0.491). The BMI of patients was (28.7±6.1 year) which was significantly higher than that BMI of control was (24.6 ± 4.1 year) (p = 0.001) as shown in figure-1.

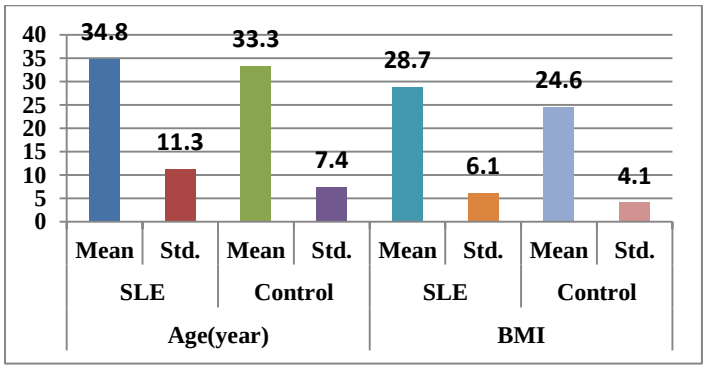

Figure 1: Age distribution and BMI.

The mean of anti-CarP antibody of SLE group (2.69±0.8) was significantly higher than that of control group (1.23±0.4) (P =0.001) as illustrated in figure-2.

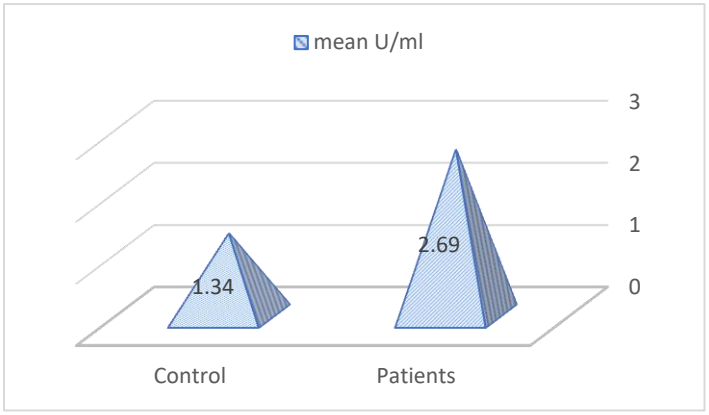

Figure 2: Differences in means of serum levels of anti–CarP antibody concentrations between SLE patients and healthy controls.

The area under the curve (AUC) was 0.858 with optimum cut off value for anti carP (2.032) to differentiate SLE patients from healthy controls. Regarding anti carP antibody, the sensitivity, specificity and accuracy of anti carP in identifying patients and controls SLE was 80%, 77.50% ,78% respectively. The positive predictive value (PPV) was 78% which means among those who had SLE, the probability of disease was 78%. while negative predictive value (NPV) was 79.50%, that means among those that do not have SLE, the probability of being disease –free was 79.50%. Figure (3) and table (1).

Table 1: Validity parameters of anti-CarP antibody to differentiate SLE patients from healthy controls.

Variable	UAC	SE	P value	Optimum cut off point	Sensitivity	specificity	PPV	NPV	Accuracy
Anti-CarP antibody	0.858	0.043	0.001	2.032	80%	77.50%	78%	79.50%	78%

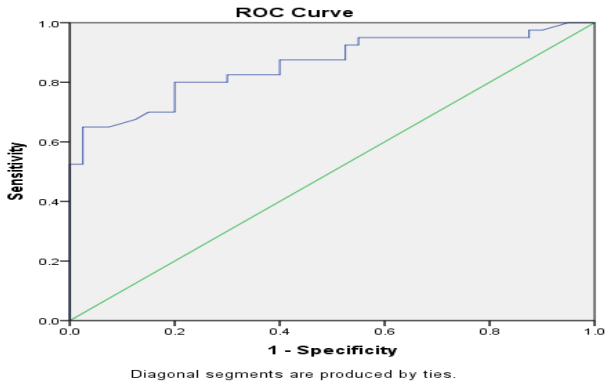

Figure 3: ROC analyses for anti-CarP antibody to differentiate SLE patients from healthy controls.

AUC: area under curve; PPV: positive predictive value; NPV: negative predictive value; SE: standard error; P < 0.05 significant

Furthermore, there was a significant association between positive result of ds-DNA antibody and positive anti-CarP antibody. (P-V =0.014) as shown in table- 2.

Table 2: Association between the laboratory findings and anti-CarP antibody.

Laboratory Tests	Results	Anti-CarP antibody				P value
		Positive		Negative		
		N	Raw %	N	Raw %	
ESR	normal	11	73.3%	4	26.7%	0.414
	high	21	84.0%	4	16.0%	
Hb	normal	28	77.8%	8	22.2%	0.292
	low	4	100.0%	0	0.0%	
WBC	normal	29	78.4%	8	21.6%	0.368
	low	3	100.0%	0	0.0%	
Blood urea	normal	30	78.9%	8	21.1%	0.468
	high	2	100.0%	0	0.0%	
Serum Creatinine	normal	31	79.5%	8	20.5%	0.613
	high	1	100.0%	0	0.0%	

A significant association was also found between serum levels of anti-CarP antibody and the serum levels of ds-DNA antibody among SLE patients with P-V =0.003 as depicted in table -3.

Table 3: Association between anti-CarP antibody and serum level of Anti ds-DNA antibody among SLE patients.

Variable	Anti-CarP antibody	N	Mean U/ml	SE	P value
Anti ds-DNA antibody	Positive	32	52.92	12.37	0.003
	Negative	8	12.97	1.46	

On the other hand, mean serum levels of anti-CarP antibody according to severity of SLE showed no significant difference (P-V =0.472) as clarified in Table- 4.

Table 4: Differences between serum levels of anti-CarP antibody in SLE patients according to severity of the disease.

SLE severity	N	Mean U /ml	SD	P-value
Mild	5	2.8	0.45	0.472
moderate	20	2.55	1.63	
High	13	2.62	1.41	
very high	2	4.30	2.13	
Total	40	2.69	1.48	

DISCUSSION:

In the current study, we found that the levels of serum anti-CarP antibodies in the patients with SLE were significantly higher than those in the healthy controls with a p value less than 0.001. Similar to this finding what was reported by Li Y et al., 2020[3] and Li YN et al., 2019[4] that reported higher serum levels of anti-CarP antibody in SLE patients than healthy controls and showed higher sensitivity and specificity for the disease.

Regarding the socio-demographic characteristics, the mean age among patients was 34.8 ± 11.3 years which was comparable to other local Iraqi studies conducted by (Dua'a et al., 2020; Ikram et al., 2020) showed that the mean age of the SLE patients was (30.23 ± 10.25, 31.91 ± 7.49 years respectively)[5,6]. Also, the mean age was comparable to a pooled analysis of 3,273 patients among Arabic countries (Egypt, Iraq, UAE, Saudi Arabia, Jordan, Kuwait, Lebanon, Oman, Sudan, Tunisia, and Yemen)[7] which showed the mean age was 28.9 years at diagnosis, while UK the mean age was (48.5 years) higher than the results of present study[8].

In the current study, all of SLE patients were females, this result was compatible with other tudies conducted in Iraq included AlHusseini's 2020[9]. This female predominance is mainly due to estrogen influence[10].

Regarding BMI among patients was 28.7 ± 6.1 kg/m2 and there was a positive correlation between anti-CarP antibody and the BMI, which indicates that patients with SLE were overweight, and the obesity has been proven as a risk factor for developing and aggravating SLE[11].

Concerning the laboratory investigations, there was a strong positive significant correlation between anti-CarP antibody and the ds-DNA antibody that is approved by the study done in China by Li YN et al.,2019[4], and the ds-DNA antibody considered as a good marker of disease activity in SLE patients[12,13]. These results demonstrated that anti-CarP antibody could play as an important marker for monitoring or predicting disease activity among SLE patients This finding could be implicated in future studies. On the other hand, most patient with positive anti-CarP antibody had high ESR and all SLE patients with positive ds-DNA antibody had positive anti-CarP antibody levels, although there was no significant correlation between anti-CarP antibody and ESR in this study that disagree with the study done by Li Y et al.,2020[3] and the study done by Li YN et al.,2019[4] this may be due to a small sample size.

In this study, there was no positive correlation between systemic lupus erythematosus disease activity index (SLEDAI)score with anti-CarP antibody titers that was approved with the study done by Özdemir B et al.,2021 [14] and Massaro L et al.,2018[15] that demonstrate there was no correlation between anti-CarP antibody and SLEDIA. It is possible to speculate that myeloperoxidase formed from NETosis may locally establish a milieu that is pro-carbamylated, which would result in the breakdown of immunological tolerance and the development of antibodies against carbamylated proteins. A crucial part of the pathophysiology of SLE is NETosis, or particular neutrophil death. Several studies have demonstrated elevated NETosis in SLE patients, and this has a strong correlation with disease activity[15].

CONCLUSION:

Serum anti-CarP antibody level was able to differentiate between patient with SLE and healthy control with sensitivity 80% , specificity 77.50% and accuracy 78%. There was a significant association between anti-CarP antibody and ds-DNA antibody levels among SLE patients therefore it could aid in predicting disease activity.

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