Prevalence of Anticitrullinated Cyclic Peptide in Systemic Lupus Erythematosus: Relation with Clinical and Laboratory Parameters.

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Abstract: Background: Systemic Lupus Erythematosus (SLE) patients may have joint complaints in early stages of the disease. Recently, it has been found that some lupus patients may have anti-cyclic citrullinated peptide antibodies (anti-CCP) in association with joint symptoms. **Aim of study:** to evaluate the prevalence of anti-CCP antibodies in SLE patients and to detect its relation with various clinical and laboratory parameters. **Patients and methods:** Fifty SLE patients were subjected to musculoskeletal examination, routine laboratory testing and immunological profiling. ELISA was done for detection of anti-CCP antibodies and interpretation of the results: Anti-CCP antibodies (EU/ml): Negative: < 20, positive >20. Plain x-ray hand and wrist was done for detection of bony erosions. **Results:** The patients were divided into 2 groups depending on their serum level of anti-CCP: Group (1): Anti-CCP negative group including 36 patients (72%) and Group (2): Anti-CCP positive group including 14 patients (28%). Comparison between both groups showed a highly significant difference (P<0.001) as regards prevalence of arthritis, Rheumatoid Factor and Erosions. **Conclusion:** Anti-CCP positivity in SLE patients is strongly associated with arthritis. Erosive arthritis in SLE tends to be of higher frequency than previously detected specially in the presence of Anti-CCP. Anti-CCP antibodies represent a promising biomarker in SLE patients predicting the presence of arthritis and may play a role in pathogenesis as regards the development of erosions in these patients.

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1.Introduction:

Systemic lupus erythematosus (SLE) is a prototype of autoimmune diseases affecting predominantly women. It is characterized by a multisystem organ involvement because of dysregulation of self-reactive B cells leading to autoantibody production, immune complex deposition and complement activation with tissue damage [1].

Among the auto antibodies described in recent years, antibody recognized epitopes that contain the amino acid citrulline, which is generated post-translationally from arginine by the enzyme peptidylarginine deiminase. These antibodies are therefore now generally called anticitrulline antibodies or anticitrullinated cyclic peptide (anti-CCP) [2].

While Anti-CCP antibodies are known as highly sensitive and specific serological markers for diagnosis of rheumatoid arthritis (RA) [3], they were found to be positive in about 10-15% of SLE patients [4]. However, the clinical significance of these antibodies in SLE is not yet well established [5]. In addition, patients with SLE in the early stages of the disease may have joint manifestations that are similar to those found in early RA to the extent that some patients may be misdiagnosed to have RA [5]. Moreover, a small number of those patients may progress to have an erosive disease. An overlap between RA and SLE known as "Rhupus" was also reported still it is rare and debatable [6]. Different previous studies have found an association between this pattern of arthritis and the presence of Anti-CCP antibodies in patients with SLE [7-9].

The aim of this work is to study the prevalence of anti-CCP antibodies in SLE patients and to detect its relation with various clinical and laboratory parameters of the disease specially arthritis and erosions.

2. Patients and Methods

The present study is a cross-sectional one that was conducted on 50 female SLE patients, diagnosed according to the revised American college of Rheumatology criteria for diagnosis of SLE [10]. Informed consents were obtained from all participants and the study was approved by the Medical ethics committee.

All patients were subjected to the following:

- **History taking and thorough clinical and musculoskeletal examination.**

- **Laboratory and immunological testing:**

*Complete blood count using automated hematology analyzer.
*Erythrocyte sedimentation rate (ESR). (Westergren method, 1st h).
*C-reactive protein (CRP) (quantitative value).
*Liver and Kidney function tests.
*Urine analysis and 24 hrs urinary protein.
*Antinuclear antibodies (ANA) and anti-double stranded (Anti-dsDNA).
*Anti phospholipid antibodies (aPL ab).
*Rheumatoid factor (RF).
*Auto antibodies to anti-cyclic citrullinated protein antibodies (anti-CCP):

By Enzyme-linked immunosorbent assay for detection and quantitation of IgG antibodies to Cyclic Citrullinated Peptides (CCP) in human serum or plasma Test principle: The microtiter plate provided in this kit has been pre-coated with an antibody specific to anti-CCP. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for anti-CCP and Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB (3, 3'5, 5' tetramethyl-benzidine) substrate solution is added to each well. Only those wells that contain anti-CCP, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of anti-CCP in the samples is then determined by comparing the O.D. of the samples to the standard curve. Interpretation of the results: **Anti-CCP antibodies (EU/ml): Negative: < 20, positive >20**

- **Radiological evaluation**

Plain X ray of the hands and wrists

Statistical methods:

Data were statistically described in terms of mean ± standard deviation (± SD), range or frequencies (number of cases) and percentages. Chi-square test was used to compare qualitative variables. Unpaired t-test was used to compare two independent groups as regard a quantitative variables. Spearman & Pearson Correlation co-efficient rank test was used to rank different variables against each other's positively or inversely. *P* value = level of significance where *P* >0.05=not significant (NS), *P* <0.05=significant (S) and *P* <0.001= highly significant (HS).

3. Results:

3.1. Demographic, Clinical and laboratory data of SLE patients

This study included fifty female SLE patients, their ages ranged from 22 - 54 with a mean of 30.16 ± 9.2 years, disease duration ranged from 18 to 223 months with the mean of 65.8 ± 34.3 and disease activity by SLEDAI ranging 4 to 72 with the mean of 21.13 ± 18.44. CRP ranged from 3 to 96 with the mean 23.52 ± 30.45.Only one patient fulfilled both SLE & RA criteria and was labeled as "rhupus". Various clinical manifestations and their percentages are shown in Table 1. Laboratory findings and presence of erosions are shown in Table 2.

Table (1): Clinical manifestations of the patients:

Manifestation	Number of patients	Percentage (%)
Musculoskeletal involvement		
Arthritis	30	60
Arthralgia	40	80
Mucocutaneous manifestations		
Skin	36	72
Oral ulcers	30	60
Renal Disease		
Nephrotic	18	36
Nephritic	13	26
CNS involvement		
Seizures	1	2
Psychosis	1	2
Respiratory system involvement		
Pleurisy	9	18
Interstitial lung disease	1	2
Pleural effusion	3	6
Vascular manifestation		
Raynaud's	13	26
Deep venous thrombosis	1	2
Mesenteric vascular occlusion	0	0
Recurrent abortions	6	12

Table (2): Laboratory and Radiological findings of the patients:

Finding	Number of patients	Percentage (%)
Autoimmune hemolytic anemia	7	14
Thrombocytopenia	5	10
ESR	44	88
CRP	22	44
ANA	48	96
Anti-dsDNA	49	98
aPL	14	28
RF	9	18
Anti-CCP	14	28
Erosions	6	12

ESR = Erythrocyte sedimentation rate, CRP = C-reactive protein, ANA= anti-nuclear antibody, Anti-dsDNA= anti-double stranded DNA, aPL: anti-phospholipid antibody, RF = rheumatoid factor, Anti-CCP = anti cyclic citrullinated peptide

3.2 Comparison between the two groups of SLE patients

The Anti-CCP results ranged from 2 to 50 with a mean of 30.35 ± 13.7 U/ml. The 50 patients were classified into 2 groups depending on their serum level of anti-CCP with cut off value 20 units. **Group (1):** Anti-CCP negative group including 36 patients (72%) and **Group (2):** Anti-CCP positive group including 14 patients (28%). Comparison between both groups as regards age, disease duration and activity (19/36 patients i.e. 52% in group 1 versus 7/14 patients i.e. 50% had SLEDAI>8 denoting active SLE) showed no statistical difference. Comparison between the two groups as regards other studied parameters shown in Tables 3 & 4.

Table (3): Comparison between the clinical manifestations of both groups:

Manifestation	Group 1 N	Group 1 %	Group 2 N	Group 2 %	*P*	Sig
Musculoskeletal						
Arthritis	19	52	11	79	*P*<0.001	HS
Arthralgia	27	75	13	93	*P*>0.05	NS
Mucocutaneous						
Skin	23	63	13	92	*P*<0.05	S
Oral ulcers	20	55	10	71	*P*>0.05	NS
Renal						
Nephrotic	10	27	8	57	P<0.05	S
Nephritic	7	25	6	28	P>0.05	NS
CNS						
Seizures	1	2	0	0	P>0.05	NS
Psychosis	1	2	0	0	P>0.05	NS
Respiratory						
Pleurisy	6	16	3	21	P>0.05 P>0.05	NS
Interstitial lung disease	1	2	0	0	P>0.05	NS
Pleural effusion	2	5	1	7		NS
Vascular						
Raynaud's	9	25	4	28	P>0.05	NS
Deep venous thrombosis	1	2	0	0	P>0.05	NS
Mesenteric occlusion	0	0	0	0	P>0.05	NS
Recurrent abortions	4	11	2	14	P>0.05	NS

Table (4): Comparing the laboratory and radiological findings between both groups:

Finding	Group 1 N	Group 1 %	Group 2 N	Group 2 %	*P*	Sig
Hemolytic anemia	5	13	2	14	*P*>0.05	NS
Thrombocytopenia	2	5	3	21	*P*<0.05	S
High ESR	32	88	12	85	*P*>0.05	NS
High CRP	11	30	11	78.5	P<0.05	S
ANA	34	94	14	100	P>0.05	NS
Anti-dsDNA	36	100	13	92	P>0.05	NS
aPL	9	25	5	35	P>0.05	NS
RF	3	8	6	42	P<0.001	HS
Erosions	2	5	4	28	P<0.001	HS

ESR = Erythrocyte sedimentation rate, CRP = C-reactive protein, ANA= anti-nuclear antibody, Anti-dsDNA= anti-double stranded DNA, aPL: anti-phospholipid antibody, RF = rheumatoid factor.

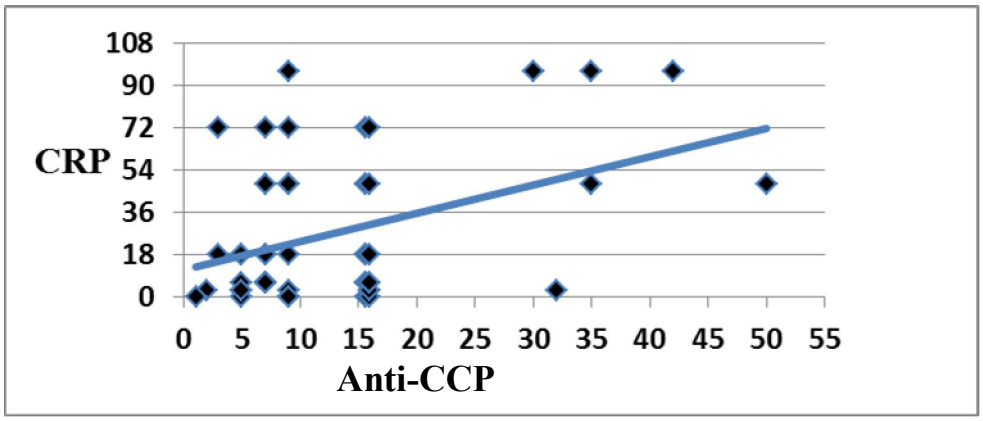

Figure 1. Correlation between Anti-CCP and CRP of the studied SLE patients.

3.3 Correlation between Anti-CCP and CRP in SLE patients

Anti-CCP showed statistically significant positive correlation with CRP (r=0.4, $P<0.05$) see figure 1. No other significant correlation has been found with other studied laboratory parameters.

4. Discussion

Systemic Lupus Erythematosus (SLE) patients may have joint complaints in the early stages of the disease. Recently, it has been found that some lupus patients may have anti-cyclic citrullinated peptide antibodies (anti-CCP) in association with joint symptoms although the clinical significance of such finding is not well established [5].

In addition, a small number of SLE patients develop an erosive disease. Some studies have suggested an association between anti-CCP antibodies and this pattern of arthritis, but their exact significance in SLE patients remains unclear [6].

The aim of this work was to study the prevalence of anti-CCP antibodies in SLE patients and its association with clinical and laboratory parameters specially arthritis. The study included 50 female SLE patients between the ages of 22-54 with disease duration ranging from 18 - 223 months.

The Anti-CCP prevalence among SLE patients in previous different studies was extremely variable. Qing et al., 2009 reported similar results to ours as they found that anti-CCP positivity was 27.3% in their SLE patients [11]. These results are similar to the results of the present study as anti-CCP antibodies were detected in 28% of our patients with SLE. Higher prevalence was reported in the study of Singh et al 2010 as Anti-CCP was tested positive in 22 out of 58 SLE patients (37.93%) but they had higher percentage of patients with arthritis 44 (75.86%) in their study than in ours 30(60%)[12]. Furthermore, a Brazilian study detected anti-CCP antibodies in 13.7 % of patients with SLE [5] and another Chinese study stated that the prevalence of anti-CCP was 13.8% (19/138) in SLE patients [13]. The cause of this variability may be due to the different ethnicities, also the variability between the SLE patients in the different studies should be considered.

In Contrast however, one study on 34 SLE patients stated that SLE patients, with or without deforming arthropathy, had normal serum anti-CCP concentrations [14]. Another study reported that anti-CCP antibodies were not even detected in 20 SLE patients with arthritis [15].

The patients in our study were divided into 2 groups depending on their serum level of anti-CCP: **Group (1):** Anti-CCP negative group including 36 patients (72%) and **Group (2):** Anti- CCP positive group including 14 patients (28%).

In the present study, Anti-CCP positive patients (group 2) exhibited significant difference in comparison to negative patients (group 1) as regards arthritis (79 vs 52%) and the presence of erosions (28 vs 5%) and presence of high CRP (78 vs 30%).

Previous studies have detected similar results, one retrospective medical record review of a case series of five female patients with SLE and erosive arthropathies detected anti-CCP antibodies in 4 out of 5 (80%) patients they concluded that erosive arthritis was strongly associated with the presence of anti-CCP antibodies [16]. Another study also detected association of anti-CCP antibodies with erosive arthritis in SLE patients (60 vs. 0%, $P = 0.02$) and concluded that high levels of anti-CCP may be a useful serological marker for an erosive arthritis pattern among these patients [17]. In addition, Zhao *et al.,* 2009 found that the frequency of arthritis in anti-CCP positive SLE patients (73.7%) was

significantly higher than in anti-CCP negative patients (47.1% , P = 0.031), they also noticed significantly higher incidence of erosive arthritis among their anti-CCP positive SLE patients with arthritis than in anti-CCP negative patients with arthritis (5/14, 35.7% vs 3/56, 5.4% , P = 0.001) [13].

Moreover, Qing *et al.,* 2009 reported that anti-CCP antibodies were more prevalent in SLE patients with arthritis than those without arthritis and concluded that high anti-CCP antibodies titer might be used as a predictor for erosive arthritis in patients with SLE [16].

In another study, 104 patients with SLE were evaluated for arthritis and classified as erosive arthritis, non-erosive arthritis, or no arthritis. Eight patients (8%) were anti-CCP positive and they accounted for 11% (8/71) of patients with synovitis. Among patients with synovitis, erosive arthritis was associated with anti-CCP (OR 28.5, 95% CI 4.7-173.8, P = 0.001) [9]. Similarly, in another study Anti-CCP was found in 17% (55/329) of those with SLE and was more common in SLE patients with deforming/erosive arthritis (38%) [4].

Although, one study did report that even though the prevalence of anti-CCP was higher in SLE patients in comparison to control yet no relationship could be found with clinical profile including joint complaints [5] and another study found no difference between the patients with or without arthritis [18].

Kakumanu *et al.,* studied the citrulline dependence of Anti-CCP antibodies and concluded that the citrulline dependent antibodies are associated with erosive or deforming arthritis in SLE patients. This together with high levels of anti-CCP would be helpful in predicting erosive arthritis in SLE [4].

Zhao *et al.,* who studied 138 patients with SLE, found statistical significant correlation between anti-CCP and CRP has been detected in SLE patients with arthritis [13]. Similarly, we demonstrate in the present study that anti-CCP positive patients with SLE show higher CRP and RF positivity than those with negative anti-CCP and in group 2 (anti-CCP positive patients), anti-CCP antibodies significantly correlated with CRP ($r=0.4$, $p<0.05$).

Interestingly, since CRP does not usually increase with disease activity in SLE, in contrast to the majority of inflammatory diseases, an increase in CRP could help to distinguish SLE patients with or without classic articular involvement. This simple marker in SLE patients with articular involvement could be a warning signal of worse articular evolution.

Also, our findings are supported by the results of Taraborelli *et al.,* 2012, as they found that RF positivity and increased CRP were more frequent in erosive arthritis than in non-erosive arthritis. This study supports the evidence that anti-CCP antibodies could be a useful marker of erosive disease in SLE patients and the increase in RF and CRP could be an additional means of identifying lupus patients with arthritis at risk of a worse prognosis [6].

Finally, in our study the prevalence of anti-CCP antibodies was significantly higher in SLE patients with arthritis, and moreover patients with erosive arthritis had a higher prevalence of anti-CCP antibodies.

In conclusion Anti-CCP positivity in SLE patients is strongly associated with arthritis. Erosive arthritis in SLE tends to be of higher frequency than previously detected specially in the presence of Anti-CCP. Anti-CCP antibodies represent a promising biomarker in SLE patients predicting presence of arthritis and may play a role in pathogenesis as regards the development of erosions in these patients. Further studies are needed to reveal the exact clinical value of this marker in SLE patients.

5. Conflict of interest

There is no conflict of interest of the authors.

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