

Serum Anti-Cyclic Citrullinated Peptide (Anti_CCP) Antibody Level in Rheumatoid Arthritic Patients with and without Nodal Osteoarthritis

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ABSTRACT:

BACKGROUND:

Rheumatoid Arthritis (RA) is chronic systemic inflammatory disease affects synovial joints , articular structures and extra articular , the prevalence rate occurs aprox in about 1% of population Anti citrullinated peptide/protein antibody (Anti-CCP) antibody are autoantibodies(antibodies directed against one or more of the individuals own proteins) frequently detected in RA patients during inflammation. citrulline is incorporated enzymatically into proteins .

The current study is a trail to ensure that Anti-CCP marker is more specific and sensitive for diagnosis of RA patients and to detect the effect of the presence of other disease like NOA on Anti-CCP value

OBJECTIVE:

Evaluation of Anti-CCP Ab level in RA patients with and without NOA.The serum level of Accp_Ab is estimated using ELISA technique in patients with RA and NOA.

SUBJECTS AND METHODS:

The studied group includes 30 RA patients (24 females and 6 males), who fulfilled four or more of the 1987 ACR criteria compared with 30 RA+NOA patients (23 females and 7 males) also fulfilled four or more of the 1987 ACR criteria for the classification of Rheumatoid arthritis and nodal osteoarthritis group. Blood samples were taken from three groups to analyze and measure the serum levels of Anti-CCP.

RESULTS:

Result of investigations were compared with that for the 30 apparently healthy control individuals who matched the patient's group in age and sex.

The result of the study showed that majority of patients is females (80%) for RA patients and (76.6%) for RA+NOA patients (with females: males 4:1 & 3.28:1 for RA and RA+NOA respectively). Moreover, the mean age for RA, RA+NOA and control groups are: 48.03±12.95y, 51.3±11.8y and 37.43±12.57years respectively.

It was observed from the result that there is a higher positivity of Anti-CCP in the patients sera(76.7% for RA+NOA cases and 70% for RA cases) in comparison with healthy control group (0.0%) so there is highly significant difference ($p<0.001$).

While the quantitative estimation of Anti-CCP antibodies showed that its level was higher among sera of RA+NOA patients in addition to RA patients (53.59±33.29 RU/ml and 52.28±48.99 RU/ml respectively) in comparison with healthy control group 2.88±2.50 RU/ml.

CONCLUSION:

We found that Anti-CCP is a good & specific marker for diagnosis of RA & RA can be differentiated from other disease by measuring this marker.

Moreover, Anti-CCP Ab level in RA patients does not significantly affected by the presence of NOA.

KEYWORDS: rheumatoid arthritis, anti-ccp, nodal osteoarthritis

INTRODUCTION:

Rheumatoid arthritis (RA) is a systemic

autoimmune disease characterized by chronic inflammation of the joints, with eventual erosive changes and joint deformities⁽¹⁾. The diagnosis of RA is primarily based on clinical manifestations of the disease, supported in many cases by serologic findings. Rheumatoid factor is present in up to 70% of patients but is also detected in a

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variety of autoimmune disorders, other non rheumatic conditions, and in healthy individuals⁽¹⁾. According to the specific test used, antibodies directed toward citrullinated proteins have been described in 60–80% of patients with RA with a specificity of 85–95%^(2,3). Citrullinated proteins seem to originate in the synovium⁽⁴⁾, and anti-cyclic citrullinated proteins (anti-CCP) appear to be produced in the inflamed synovium by local plasma cells⁽⁵⁾.

Furthermore, this chronic disease characterized by synovial inflammation and subsequent tissue damage⁽⁶⁾. It affects approx 0.5-1% of world's population⁽⁶⁾. It is associated with reduced life expectancy and is a major cause of chronic disability and handicap⁽⁷⁾. Hypertrophy and inflammation of the soft tissues around synovial joints is the common phenomenon occurring in RA. The etiology of RA is not known but it is classified as one of the autoimmune disease⁽⁷⁾. Osteoarthritis is a degenerative articular disease with a complex pathogenesis because of diverse factors interacts causing a process of deterioration of the cartilage and the subchondral bone. It can be primary or secondary to divers diseases, but it has clinical, radiological, and pathological manifestations in common.

Osteoarthritis is considered as a heterogeneous group, with common and different aspects;⁽⁸⁾.

The hand is commonly involved, and polyarticular interphalangeal OA is taken as the marker for predisposition to OA at multiple sites (generalised OA)⁽⁹⁾. Nodal OA (NOA), a subset of OA, is characterized by polyarticular interphalangeal and thumb base OA, Heberden's and Bouchard's nodes formation, a predominance in women, and a clear genetic predisposition.

Osteoarthritis can be localized on one, two, or more joints, but if it affects three or more joint groups, it is usually known as polyarticular or generalized OA⁽¹⁰⁾. The estimated prevalence of OA in the hands varies depending on the definition⁽¹¹⁾. Although the prevalence of radiographic OA is reported to be as high as 29-76% in population based studies in subjects with age more than 55 years, the prevalence of symptomatic hand OA is much lower, the prevalence of OA, usually, increases with age⁽¹¹⁾.

SUBJECTS AND METHODS:

The study was included on (90) subjects, (30) of them with RA only who fulfilled four or more of the 1987 ACR criteria compared with (30) RA+NOA patients also fulfilled four or more of

the 1987 ACR criteria for the classification of Rheumatoid arthritis and nodal osteoarthritis group and (30) age and sex matched healthy control.

The study performed during the period from December 2009 to April 2010. These subjects were selected from patients attending the outpatient clinic in Medical City-Baghdad Teaching Hospital – Rheumatology Consultation Unit, where the anthropometric tests were performed. The other tests were done in Medical City – Teaching Laboratories and the college of Medicine – Department of Physiological Chemistry. Three study groups were investigated which included:

*First group: patients with rheumatoid arthritis only who fulfilled the 1987 ACR criteria for RA (RA ONLY) group.

*Second group: patients with rheumatoid arthritis and NOA according to 1990 ACR criteria for NOA (RA-NOA).

*Third group: Healthy control group who had no history or clinical evidence of RA or any other chronic disease.

Immunological tests:-Detection of serum Anti-CCP by enzyme-linked immunosorbent assay (ELISA).

Resultes above 25 U / ml (cut-off value) are considered positive, statistical tables including observed frequencies with their percentages. Summary statistics of the readings distribution (mean±SEM). Graphical presentation by (bar-charts). These were used to accept or reject the statistical hypotheses, Chi-Square (χ^2), Student test (t-test), ANOVA test (F-test) & LSD test (F-test). The comparison of p-value significance (Sig.) in any test was: S=Significant difference (p<0.05). HS=Highly Significant difference (p<0.01). NS= Non significant difference (p>0.05). The statistical analyses were done through the Statistical Package for the Social Sciences (SPSS) program (version-10) and Excel application.

RESULTS:

The demographic picture of studied group reveals that the majority of RA and RA+NOA patients are females with female: male ratio 4:1 & 3.28:1 respectively. While the frequency of females among healthy control is (70%) with female: male ratio of 2.3:1. Moreover, the mean age for RA, RA+NAO and healthy control groups are (48.3±12.95), (51.3±11.8), (37.43±12.57) respectively. It appears that there is no significant difference between RA and

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RA+NOA groups regarding disease duration as (9.67 ± 6.57) and (7.66 ± 9.63) years respectively ($p=0.35$). The Rheumatoid factor in the patients' sera represent one of the rheumatoid arthritis markers. It has been detected by using qualitative and quantitative screening test. The RF value in the current study shows 66.7% positivity for RA cases and 70% positivity for RA+NOA. The newly diagnostic marker for RA

is anti-CCP antibody which has been detected in the sera of the studied groups. The results of its frequencies among the studied groups are listed in Table (1&2) and figure (1). The highly frequency of anti-CCP positivity that occurs among studied groups was in RA+NOA group (76.7%) and (70%) for RA group, While its (0.0%) for control group. So is a good indication for its specificity for RA disease

Table 1: Distribution of Anti-CCP (RU/ml) among studied group.

Studied group		Anti-CCP		Total	Comparison of Significant	
		Positive	Negative		P-value	Sig.
RA	N	21	9	30	0.00	Highly Sig. ($p < 0.01$)
	%	70	30	100		
RA+NOA	N	23	7	30		
	%	76.7	23.3	100		
Control	N	0.0	30	30		
	%	0.0	100	100		
Total	N	44	46	90		
	%	48.9	51.1	100		

Table 2: Mean distribution of Anti-CCP level (Relative Unit/ml) among studied group.

Studied group	N	Mean	Std. Deviation	ANOVA test	
				P-value	Sig.
RA	30	52.28	48.99	0.00	Highly Sig. ($P < 0.01$)
RA+NOA	30	53.59	33.29		
Control	30	2.88	2.50		
Total	90				

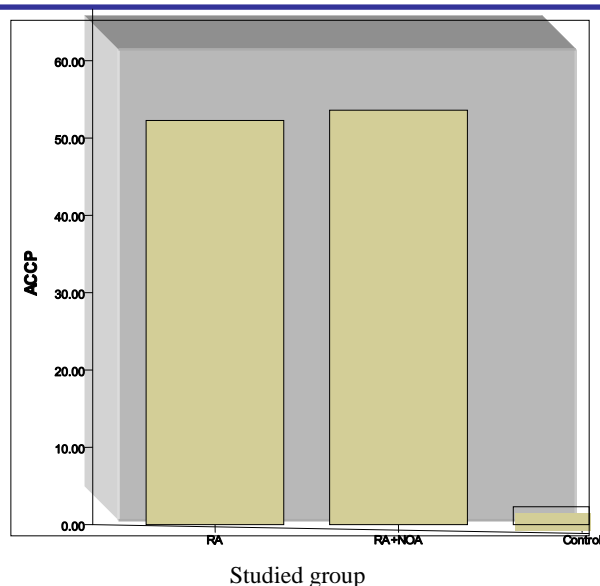


Figure 1: Distribution of Anti-CCP (RU/ml) among studied groups.

Table 3: Correlation between Anti-CCP & Other parameter among RA&RA+NOA

Parameter		Anti-CCP	
		RA	RA+NOA
CRP	R	0.154	-0.066-
	P-value	0.416	0.729
RF screening test	R	0.859**	0.879**
	P-value	0.000	0.000
DAS28	R	0.228	0.039-
	P-value	0.226	0.838
ESR	R	0.050	0.129-
	P-value	0.794	0.498

DISCUSSION:

Anti-CCP marker had been reckoned as a diagnostic marker characterized with all these features as proved by the results of the current study (76.7%) positivity for RA+NOA and(70%) for RA cases inversely to (0.0)% for healthy control).

From the result we found that NOA disease that accompanying to RA does not affect the Anti-CCP result significantly that indicate Anti-CCP Ab are specific to RA and do not affected by the presence of other disease like NOA and the result in agreement with G-Morozzi *et al.*,(2005)^(12,13) that reported Anti-CCP positivity is(0.0) in NOA.

found that Anti-CCP & RF is negative in NOA patients.

The specificity of anti-CCP in diagnosing RA is

well known, and in our study it was 100%.

Citrulline is formed by de-immination of arginines residues in several proteins by the action of enzyme peptidylarginine deiminase (PAD).The isoenzymes of PAD are abundant in the inflammatory synovium and causes local citrullination of synovial proteins, such as the production of Anti-CCP and anti-flaggrin antibodies in the joints ^(14, 15,16).

According to these finding, it was observed that Anti-CCP antibodies can be detected years before manifestations of the symptoms, indicating that the initial events that lead to this autoimmune disease may have taken place years before clinical manifestation. The pre-disease activity and associated complaints are going up and down until a certain arthritis threshold is

reached. Only then, the patients will pay the first attention to visit the Rheumatologist clinic. By that time about 70% of the patients is Anti-CCP positive, Nevertheless, in most cases not all clinical parameters of RA are present and it takes on average 6-8 months as have been reported by (4) before a definitive diagnosis can be made. This lag time may be significantly reduced when Anti-CCP is included as a diagnostic criterion.

The current study demonstrated that 76.7% of RA+NOA patients & 70% of RA patients developed Anti-CCP. This result is similar to Jinan El-Saffar (2008) result that was 70% Anti-CCP positive for RA and it is higher than that of (Shankar, *et al.* 2004) ⁽¹⁷⁾ and Nell, *et al.*, (2003) ⁽¹⁵⁾ who proposed 50-60% detection of Anti-CCP in RA patients. Regarding that only high level of Anti-CCP was considered (≥ 50 RU/ml) ⁽¹⁸⁾. This explains the difference between the higher present data and those for the European populations. Moreover, many generations of the test has been applied such as Anti-CCP1 or 2 with different sensitivities which added another reason for these variations ^(19,20).

Regarding the previous findings, it seems that Anti-CCP is a good diagnostic marker since it elevated significantly in the sera of RA patients (52.28 \pm 48.99RU/ml) for RA alone and (53.59 \pm 33.29 RU/ml) for RA+NOA patients in comparison with low non-significant level of these antibodies among the control groups (2.88 \pm 2.50RU/ml) for healthy control as shown in Table (1) and Figure (1) (P< 0.001).

CONCLUSION:

We found that Anti-CCP is a good & specific marker for diagnosis of RA & RA can be differentiated from other disease by measuring this marker.

Moreover, Anti-CCP Ab level in RA patients does not significantly affected by the presence of NOA. So the presence of NOA in patients with RA does not increase RA disease activity.

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