Original Article

Correlation of hs-CRP Levels with Anti-CCP And Rheumatoid Factor Among Clinically Suspected Rheumatoid Arthritis Cases: A Predictor for Early Inflammation?

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ABSTRACT

Objective: Despite the wide availability of markers to diagnose the established disease of RA, there is lack of evidence for the suitability of any of these established biomarkers for diagnosing the disease at an early stage of its pathogenesis. Hs-CRP has the potential to be useful as a predictor of early inflammation in clinically suspected RA cases.

Methods: 80 patients (40: anti-CCP positive; 40: anti-CCP negative) irrespective of their age and gender were enrolled. RF status and hs-CRP levels were determined in these patients. Correlation of hs-CRP levels with anti-CCP was done.

Results: Mean anti-CCP levels among RF positive and negative cases were 410 U/ml and 62.4 U/ml respectively. Among both the groups of anti-CCP positive and negative patients, majority had hs-CRP levels between 50-60 mg/l. Hs-CRP levels were more than 6 mg/l in majority of the suspected arthritis cases irrespective of the anti-CCP or RF status. Mean hs-CRP levels were 50.8 and 43.6 mg/l among anti-CCP positive and negative cases respectively by unpaired t-test. There was no correlation between serum anti-CCP and hs-CRP levels among both anti-CCP negative and positive cases.

Conclusions: Hs-CRP when used alone or in combination with other established markers, can aid in the early diagnosis, prediction of course of disease and assessment of response to treatment in RA cases.

Keywords: hs-CRP; anti-CCP; RF; RA

Introduction

Rheumatoid arthritis (RA) is widely prevalent autoimmune disorder primarily involving the small joints of the hands and feet. The worldwide prevalence of RA among adults is approximately 0.5% to 1%.[1,2] RA affects women two to three times more frequently as compared to men. Uncontrolled RA may exacerbate further joint deterioration that may lead to severe disability, poor quality of life or even premature death.[3-5] RA also predisposes to several comorbid conditions like cardiovascular disease, lymphoma, lung cancer, melanoma, infectious disease.[1,2]

In current practice, the diagnosis of RA relies on the serological evidence of autoantibodies in synovial fluid and serum. Rheumatoid factor (RF) was the first autoantibody found to be associated with RA.[6] Subsequently other autoantibodies targeting autoantigens of cartilage proteins, nuclear components, enzymes and citrullinated proteins were identified.[7] RF is an IgM autoantibody directed to the Fc region of IgG. Low-affinity RF is often produced transiently in response to polyclonal B-cell activators like bacterial lipopolysaccharides and Epstein-Barr virus.[8,9] Normally RF escalates the immune complex clearance by enhancing its size and avidity. It further enhances the efficient antigen presentation to T cells along with the facilitation of complement fixation mechanisms.[10,11] However, high affinity RF in high-titers employ these actions in a pathogenic manner in the synovial fluid of RA cases. No clear evidence suggests the role of RF in initiating the disease process of RA in early stages, rather RF has been proposed to be triggered by RA.[12]

Anti-citrullinated peptide antibody (anti-CCP) constitutes another group of autoantibodies routinely used to establish the diagnosis and prognosis of RA. Amino acid citrulline is produced by the post-translational modification of arginine during inflammatory process. Citrullination of synovial proteins in RA joints induces generation of anti-CCP locally.[13] Though anti-CCP is considered a better diagnostic marker for RA than RF due to its high specificity of 95-97%,[14] the onset of anti-CCP positivity may not always coincide with onset of RA disease.[12] Anti-CCP seroconversion has been reported to precede the clinical symptoms of RA in some cases, while in others it follows the onset of RA symptoms.[15] Presence of substantial synovitis on histology even in clinically uninflamed joints

indicates the possibility of an asymptomatic or preclinical stage in RA pathogenesis. No clear evidence exists to elucidate the status of RF and anti-CCP in relation to this preclinical phase of RA.[12] Despite the limitations, RF and anti-CCP are the most frequently utilised markers for diagnosis of RA in clinical practice.

For the effective management of RA, it is crucial to detect the disease process at its earliest. High sensitivity C-reactive protein (hs-CRP) holds the potential to be useful as a surrogate marker of early RA disease. C-reactive protein (CRP) is an acute-phase reactant protein whose concentration in the blood depends on the severity of the stimuli like inflammation or trauma. CRP, a sensitive marker of systemic inflammation, can be monitored to assess the progression of an inflammatory process such as RA.[16] Hs-CRP has been recently approved for the evaluation of low disease activity in RA.[17, 18] Routine CRP testing might miss the systemic inflammation commonly associated with RA.[19] Association of poorer outcomes even with mild disease activity further suggest the role of hs-CRP in early detection of RA cases. Therefore, present study was conducted to evaluate the potential role of hs-CRP in correlation with established biomarkers of RF and anti-CCP among clinically suspected cases of RA.

Material and Methods

This study was carried out in the Immunology lab of Department of Microbiology of a tertiary care hospital of north India.Serum samples were collected from the clinically suspected cases of RA. Their anti-CCP status was assessed and a total of 80 consecutive patients (40: anti-CCP positive; 40: anti-CCP negative) irrespective of their age and gender were enrolled in the study.Serum samples from these patients were aliquoted and stored at -80°C until further assayed. RF status and hs-CRP levels were determined in these patients. Detection of anti-CCP, RF and hs-CRP was done by the commercially available kits following the manufacturers' instructions. Anti-CCP levels were determined by using enzyme-linked immunosorbent assay (ELISA) Wel-*Lisa* Anti-CCP ELISA kit (Weldon Biotech, India). This test provides the quantitative determination of anti-CCP concentrations in human serum or plasma. The OD values were generated and anti-CCP concentrations were calculated according the standard curve. RF status was assessed by the latex agglutination slide test for the detection of RF (Recombigen Laboratories, New Delhi, India). The presence of agglutination indicates the RF concentration of ≥ 8 IU/ml in the sample and lack of agglutination indicates levels below 8 IU/ml. Quantitative determination of CRP was performed by using the solid-phase ultra-sensitive enzyme immunoassay for human

serum kit (XEMA, Russia). It is based on the principle of two-site immunoassay. The sensitivity of assay was 0.05 mg/l.

Results

In present study, the age ranged from 4 years to 75 years. Majority (33.8%) of patients belonged to 30-40 years age group, followed (17.5%) by 40-50 years age group. Figure 1 shows the age and sex distribution of patients. Majority (33.3%) of males belonged to 20-30 years age group, whereas among females 30-40 years age group was the most predominant (37.1%) one. The extremes of age i.e, less than 10 years and more than 60 years, constituted 18.8% of study subjects. Female patients outnumbered the male patients with a male:female ratio of 0.29. Department wise distribution of study subjects is depicted in figure 2. 50% of patients were from Orthopaedics Department, followed by 43.8% patients from Medicine Department.

Mean anti-CCP levels among RF positive and negative cases were 410 U/ml and 62.4 U/ml respectively.Table 1 shows the distribution of rheumatoid factor according to anti-CCP positivity.The mean anti-CCP levels were 10.1 U/ml (S.D ±9.2) and 371.8 U/ml (S.D ±488.6) among the anti-CCP negative and positive patients respectively. Majority of the patients had hs-CRP levels between 50-60 mg/l. Among both the groups of anti-CCP positive and negative patients, majority had hs-CRP levels between 50-60 mg/l. Similarly, hs-CRP levels of 50-60 mg/l were also predominant among both the groups of RF positive and negative patients (Table 2). Hs-CRP levels were more than 6 mg/l in majority of the suspected arthritis cases irrespective of the anti-CCP or RF status (Table 3; Figure 3).The mean hs-CRP levels were 50.8, 43.6 mg/l amongst anti-CCP positive and negative cases respectively by unpaired t-test. There was no correlation between serum anti-CCP and hs-CRP levels among both anti-CCP negative and positive cases by Pearson correlation coefficient (rho=-0.144, p=0.376 and rho=0.104, p=0.523 respectively). No correlation was seen between serum anti-CCP and hs-CRP levels among rheumatoid factor negative and positive patients by Pearson correlation coefficient (rho=-0.103, p=0.491 and rho=0.205, p=0.253 respectively).

Discussion

RA is a chronic, disabling disease which may restrict the functional activity of involved joints unless managed early. In present study majority of patients belonged to 20-40 years age group. Our findings were in concordance with a previous study done in the same institution.[16] In our study 77.5% of patients were females. Similarly, Dessein PH *et al* in their study reported 80% of patients being women.[19] Our study observed a similar pattern of male: female ratio

Table 1: Distribution of rheumatoid factor according to anti-CCP positivity (n=80).
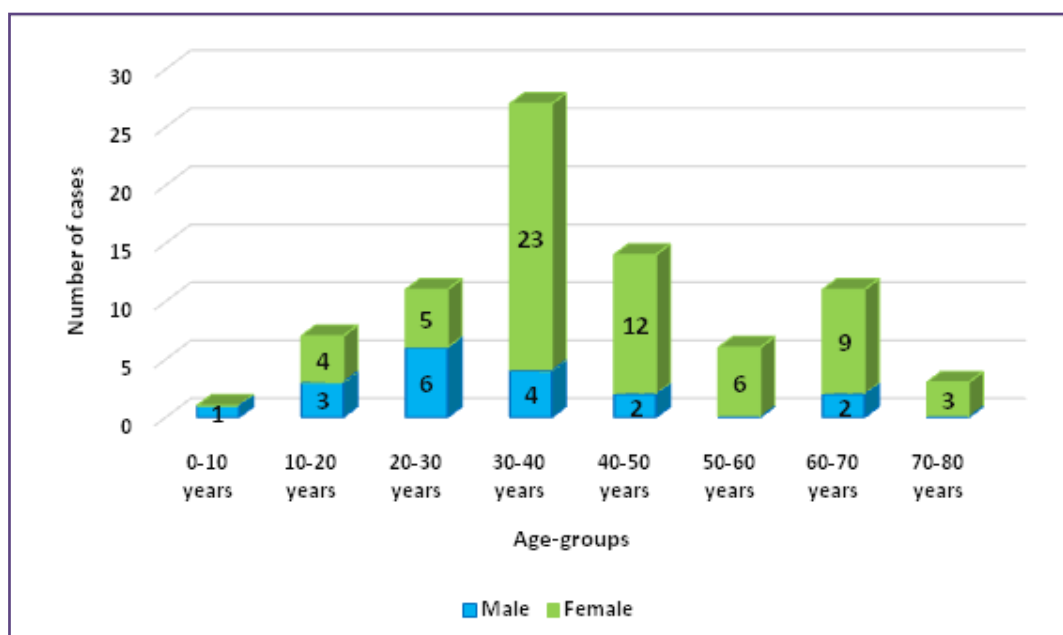
anti-CCP	Rheumatoid factor		Total
	Positive n(%)	Negative n(%)	
Positive	29 (72.5)	11 (27.5)	40
Negative	4 (10)	36 (90)	40

Table 2: Distribution of hs-CRP levels according to anti-CCP and rheumatoid factor positivity (n=80).

hs-CRP		anti-CCP		Rheumatoid factor	
	Total n(%)	Positive n(%)	Negative n(%)	Positive n(%)	Negative n(%)
0-10 mg/l	2 (2.5)	0	2 (5)	1 (3)	1 (2.1)
10-20 mg/l	3 (3.8)	2 (5)	1 (2.5)	1 (3)	2 (4.3)
20-30 mg/l	7 (8.8)	0	7 (17.5)	1 (3)	6 (12.8)
30-40 mg/l	4 (5)	1 (2.5)	3 (7.5)	1 (3)	3 (6.4)
40-50 mg/l	7 (8.8)	5 (12.5)	2 (5)	4 (12.1)	3 (6.4)
50-60 mg/l	57 (71.3)	32 (80)	25 (62.5)	25 (75.8)	32 (68.1)
Total	80 (100)	40 (100)	40 (100)	33 (100)	47 (100)

Table 3: Distribution of anti-CCP and rheumatoid factor according to hs-CRP levels (n=80).

hs-CRP	anti-CCP		Rheumatoid factor	
	Positive n(%)	Negative n(%)	Positive n(%)	Negative n(%)
≤6 mg/l	0	2 (5)	1 (3)	1 (2.1)
>6 mg/l	40 (100)	38 (95)	32 (97)	46 (97.9)
Total	40	40	33	47

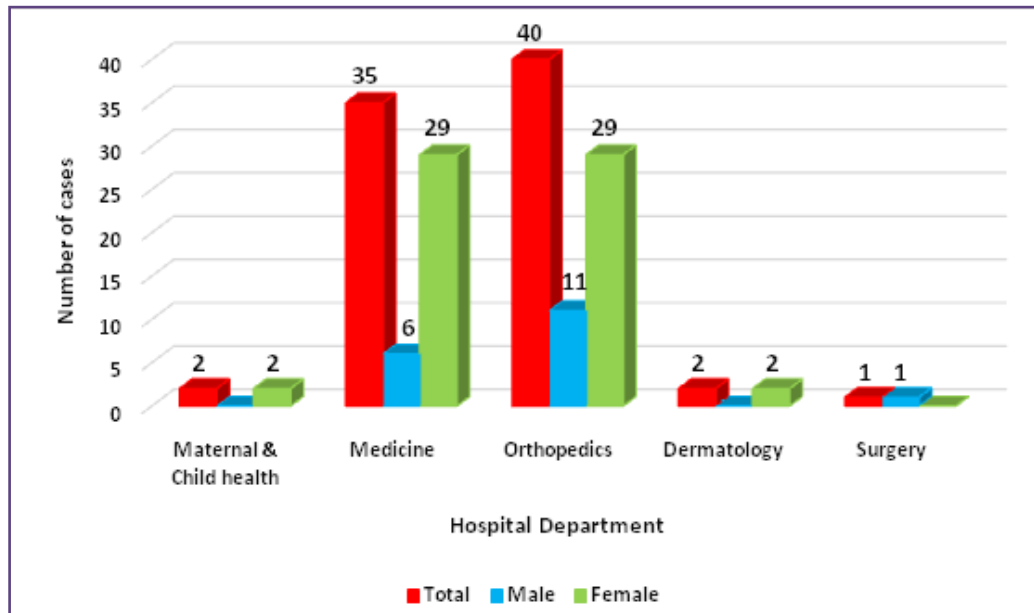Fig. 1: Distribution of age and sex among study subjects (n=80).

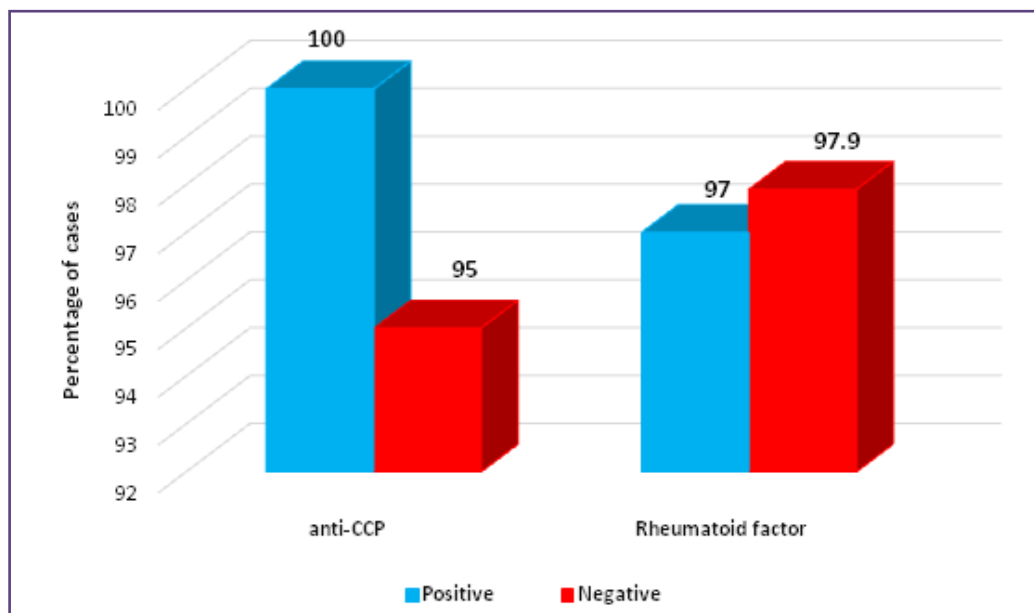**Fig. 2: Department wise distribution of study subjects (n=80).**

Fig. 3: Percentage distribution of hs-CRP levels of ≥ 6 mg/L among anti-CCP & rheumatoid factor positive and negative cases (n=78).

as another study.[16] In our study majority of patients were from Orthopaedics Department, followed by Medicine Department. Our findings were in agreement with another study.[16]

Mean anti-CCP levels among RF positive and negative cases were 410 U/ml and 62.4 U/ml respectively. In our study 27.5% of the patients with positive anti-CCP levels had negative RF levels. Shen R *et al* in their study reported

6.3% negativity among anti-CCP positive RA patients.[20] In present study RF positivity was 72.5% and 10% in anti-CCP positive and negative patients. However, Serdaroğlu M *et al* observed a higher RF positivity of 90% and 40% among anti-CCP positive and negative cases respectively.[21] In our study we have assessed the anti-CCP levels both qualitatively and quantitatively among the clinically suspected RA cases. Despite the best of our efforts, we

could not find any other research that has quantitatively assessed the anti-CCP levels in suspected or diagnosed RA cases. The review of anti-CCP levels in a patient might aid the clinician in the progression of disease or the assessment of response to treatment when the anti-CCP levels still lie in the same side of dichotomous qualitative analysis.

Hs-CRP being a sensitive indicator of systemic inflammation holds a potential to be useful as the marker to detect the early disease process in RA. Majority of the past researches are based on the routine CRP testing. The lower detection limit may vary in different settings. In our setting we use 6 mg/l as the lower detection limit for routine testing. Ridker PM *et al* in their study have reported the mean CRP levels in general population to be consistently below 2 mg/l.[22] Sharif M *et al* have demonstrated increased hs-CRP levels in 47% of the RA patients with routine CRP levels with in normal range (<10 mg/l).[17] In the present study, we observed raised hs-CRP levels (>2 mg/l) among all the clinically suspected cases of RA irrespective of their RF or anti-CCP status. The presence of consistently raised hs-CRP levels even in anti-CCP negative patients substantiate the hypothesis of initiation of disease activity much before the seroconversion for anti-CCP or RF. Furthermore RF and anti-CCP status in the preclinical phase of RA is not clearly established.[12] The presence of undifferentiated arthritis in current cases can also produce a similar scenario. In our study we observed no correlation between hs-CRP and anti-CCP among clinically suspected RA cases. Our findings are in line with the study by Serdaroğlu M *et al* that has also reported no significant correlation between anti-CCP and CRP as a marker of disease activity in RA.[21] Our study had a few limitations including the fact that we could not exclude the other common causes of raised hs-CRP levels in our patients. Though hs-CRP has been extensively studied relatively more in association with cardiovascular diseases, other co morbidities like undifferentiated forms of arthritis, non alcoholic liver disease, tuberculosis, smoking that can show increased CRP levels need to be essentially ruled out before coming to any conclusion. We need more elaborate studies involving the larger sample size with more heterogeneous study population investigating the potential role of hs-CRP in predicting the cases of RA at an earlier disease phase in comparison to the established markers of RF and anti-CCP.

Conclusion

Despite the wide availability of markers to diagnose the established disease of RA, there is a lack of evidence for the suitability of any of these established biomarkers for diagnosing the disease at an early stage of its pathogenesis. Hs-CRP may prove to be useful as a predictor of early

inflammation in clinically suspected RA cases. Detection of the disease at an early stage will help in limiting the associated functional disability. Hs-CRP when used alone or in combination with other established markers, can aid in the early diagnosis, prediction of course of the disease and assessment of response to treatment in RA cases.

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