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Anti-CCP is Associated with Greater Disease Burden in Kashmiri Population with Rheumatoid Arthritis

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Abstract

Aim: To analyse the diagnostic value of anti-cyclic Citrullinated Peptide (anti-CCP) and to associate it with disease severity in patients with rheumatoid arthritis in Kashmiri population.

Methods: The serum levels of anti-CCP levels were measured in 150 RA patients and 30 healthy controls by ELISA technique.

Result: 118 (78.66%) patients were positive for anti-CCP while none of the healthy controls were positive for the test. The mean ± SD serum anti-CCP levels were significantly ($p<0.0001$) higher in RA patients than in healthy controls (68.33 ± 42.13 U/mL vs 2.88 ± 1.81 U/mL). The sensitivity and specificity of the test was 78.67% (CI: 71.24-84.93 and 100 % (CI: 88.32-100%) respectively. The PPV and NPV of test for RA diagnosis was 100% (CI: 96.89-100%), and 48.39% (CI: 35.50-61.43%) respectively. There was a significant correlation between higher mean ± SD anti-CCP levels and female gender ($p = 0.01$), ESR > 20 mm/h ($p = 0.01$), RF positivity ($p = 0.0001$) and High DAS28 Score ($p = 0.0001$).

Conclusion: Serum anti-CCP is a very accurate marker for RA diagnosis and the patients with higher levels of anti-CCP have more aggressive form of disease. Therefore, its early detection may help clinicians in early diagnosis and appropriate management of the disease.

Keywords: Anti-CCP; Rheumatoid arthritis; ELISA

Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disorder that is characterized by chronic inflammation of the joints, with eventual erosive changes and joint deformities [1]. The diagnosis of RA is based primarily on clinical manifestations of the disease, supported in many cases by serologic findings. The serology test which is routinely used in RA is the determination of serum Rheumatoid Factor (RF) which has high and acceptable sensitivity, but modest specificity, particularly in the early course of the disease [2]. Moreover, RF is present in a many other autoimmune disorders, non-rheumatic conditions, and in healthy individuals [1]. The more recent serological test for the diagnosis of RA is anti-cyclic citrullinated peptide antibodies (anti-CCP antibodies). Citrullinated proteins originate in the synovium [3], and anti–CCP antibodies appear to be produced in the inflamed synovium by local plasma cells [4]. Studies have shown that anti- CCP antibodies test have a higher specificity and comparable or even higher sensitivity with respect to RF or other ACPA, including the recently discovered anti-mutated citrullinated vimentin antibodies for the diagnosis of RA [5]. Anti-CCP antibodies have been described to a very high level of diagnostic accuracy with a specificity of 95% to 97% and a sensitivity of 67% to 80% [6,7]. The presence of anti-CCP particularly high levels has been associated with more severe clinical outcomes, higher disease activity and worse radiographic progression in RA [8-11]. Some retrospective studies have shown the presence of anti-CCP antibodies in the serum of subjects later developing RA up to fourteen years before the first clinical symptoms [12,13]. Similar findings have been obtained in studies involving patients with early disease, thus confirming the clinical utility of anti-CCP as a diagnostic and prognostic tool in subjects presenting with RA lasting less than one or two years [5,6]. As a consequence, the new 2010 RA classification criteria have added it as one of key item in order to diagnose RA in an earlier phase [14].

We have designed our study to analyse the value and significance of anti-CCP titer with diagnosis and disease activity in patients with RA in Kashmiri population.

Materials and Methods

Study population

Our study group consisted of 150 RA patients (M/F: 131/19) fulfilling the 2010 revised criteria for the classification of RA given by American Rheumatism Association [15] and 30 (M/F: 24/6) healthy controls with negative history of any other autoimmune/inflammatory disorders were also included in the study. The cases and controls were matched for age and gender. All the patients were recruited from the Division of Rheumatology, Department of Internal Medicine, Sher-i-Kashmir Institute of Medical Sciences hospital during the period April 2012- March 2014. Data from all RA patients was obtained from personal interviews with patients and/ or guardians, and from clinical

examination. The data collected included gender, age and dwelling, RF, ESR, Swollen Joint Count (SJC), Tender Joint Count (TJC) and DAS 28 Scoring. Both the patients and controls gave informed consent to participate in the study. The study was approved by ethics committee of the institute.

Sample collection

About 5 mL of peripheral blood was collected from all the patients and healthy controls. Serum was isolated from whole blood by standard centrifugation and was stored at 4°C. Serum anti-CCP levels were dynamically measured with enzyme linked immunosorbent assay (ELISA) in both patients controls using a commercially available Human anti-CCP ELISA kit (Genesis Diagnostics, Cambridgeshire) as per the manufacturer's instructions. The concentration of < 6.25 U/mL were taken as negative for anti-CCP and levels ≥ 6.25 U/mL were taken as positive.

Calculation of DAS28

Disease activity was accessed using the DAS28 scoring. DAS28 with three variables was used, based on the counts of tender joints and swollen joints (28 joints were assessed which included 10 PIP's, 10 MCP's, 2 wrists, 2 elbows, 2 shoulders and 2 knees) and ESR. The value was calculated by using the formula DAS28 = 0.56 * √(tender joints) +0.28 * √(swollen joints)+0.70 * ln (ESR)+0.014 * Patient Global Health. Disease activity was determined as follows: DAS28 < 2.6= remission, DAS 28 < 3.2= low disease activity, DAS 28 < 5.1=moderate disease activity, DAS 28 > 5.1 = high disease activity.

Statistical analysis

The statistical analysis was performed using SPSS software, version 16 (SPSS Inc., Chicago, IL, USA). Frequency table was used to assess sensitivity, specificity, negative and positive predictive values. 95% confidence interval was calculated using the Wilson method. Receiver Operating Characteristic (ROC) curve was generated by the method of Hanley and Mclein method using XLSTAT 2015 software.

Result

Anti-CCP

The anti- CCP levels were significantly increased in RA patients as compared to healthy controls (p=0.0001). The mean anti- CCP levels were 68.33 U/mL (SD=42.13) in RA patients as compared to 2.88 U/mL (SD=1.81) in healthy controls (Table 1).

Subjects	Anti-CCP Expression (Mean ± SD)	Number	P-value
Cases	68.33 ± 42.13	150	0.0001
Controls	2.88 ± 1.81	30	
Significant p-values are shown in bold.			

Table 1: Anti- CCP levels in serum of rheumatoid arthritis cases and controls.

Based on the cut-off value suggested by the manufacturer in the RA group comprising of 150 patients, 118 sera were positive for anti-CCP at ≥ 6.25 U/ml. The sensitivity was 78.67% (CI: 71.24-84.93). In the healthy control group (30 participants), none was positive for anti-CCP. The specificity was 100 % (CI: 88.32-100%). The anti-CCP antibodies had PPV and NPV for diagnosis of RA of 100% (CI: 96.89-100%), and 48.39% (CI: 35.50-61.43%) respectively (Table 2).

	RA (N%)	Controls (N%)	Predictive values
Anti-CCP Positive	118(78.66)	0(0%)	100%
Anti-CCP Negative	32(21.33)	30(100%)	48.39%

Table 2: Relative distribution of anti-CCP results in studied patients according to RA diagnosis.

Positive predictive value; Negative predictive value; Sensitivity=78.67%; Specificity=100%

ROC curve was generated by the method of Hanley and Mclein Method for anti-CCP, and its accuracy was measured by the area under the ROC curve. The area under the curve for anti-CCP was 0.880. The accuracy was therefore considered as good (Figure 1).

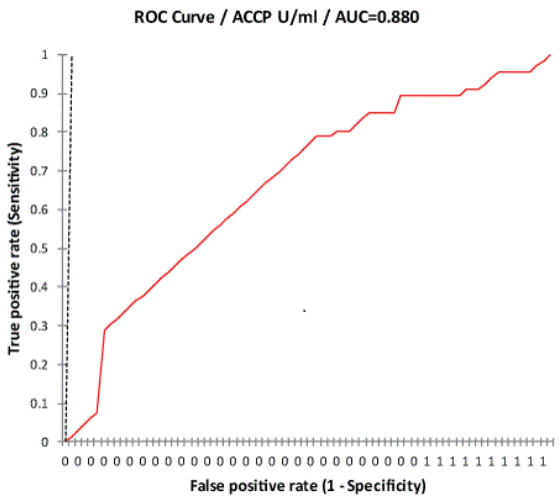

Figure 1: Receiver operating curve for anti-cyclic Citrullinated peptide in rheumatoid arthritis.

Relationship between anti-CCP with age and gender

We found that the anti- CCP levels were significantly increased in female RA patients with mean anti- CCP levels of 93.26 U/mL (SD=47.86) as compared to male patients with mean anti- CCP levels of 65.37 U/mL (SD=32.90) (p=0.01). However no association was found between the anti- CCP levels and age of patients (Table 3).

Relationship between anti-CCP and inflammatory acute phase reactants

A significant relationship was found between anti-CCP titre and ESR values (p=0.01). Patients with higher levels of ESR had increased mean anti-CCP levels (78.39 U/mL, SD=40.60) (Table 3).

Relationship between anti-CCP and Rheumatoid Factor

There was a significant correlation between the presence of RF and anti-CCP titre (p=0.0001). The mean anti-CCP in patients with positive RF was higher than in negative RF patients: 76.00 U/mL (SD 43.63) versus 37.07 U/mL (SD 41.68) (Table 3).

Relationship between anti-CCP and disease activity

A significant relationship was found between anti- CCP titre and DAS28 Score (p=0.0001). Patients with DAS28 Score > 5.1 had increased anti-CCP levels (Mean= 78.39 SD= 40.60) as compared to patients with DAS28 Score ≤ 5.1 (Table 3).

Variable	Anti-CCP Expression (Mean ± SD)	Number of Patients	P-value
Age group			
≤ 45	72.90 ± 43.67	90	0.3
> 45	64.96 ± 48.87	60	
Gender			
Female	93.26 ± 47.86	131	0.01
Male	65.37 ± 32.90	19	
RF			
Positive	76.00 ± 43.63	120	0.0001
Negative	37.07 ± 41.68	30	
ESR			
≤ 20 mm/h	60.44 ± 45.50	81	0.01
> 20 mm/h	78.39 ± 40.60	69	
DAS 28 Score			
≤ 5	50.47 ± 37.41	106	0.0001
> 5	81.75 ± 41.50	44	
Significant p-values are shown in bold.			

Table 3: Correlation of Anti-CCP levels with different clinical and laboratory parameters of Rheumatoid Arthritis cases.

Furthermore, analysing the anti-CCP level among groups with remission, low, moderate and high disease activities, a significant linear relationship was observed (p<0.05) (Table 4).

Patients in remission (DAS 28<2.6) had lowest mean anti-CCP titre of 3.67 U/mL (SD=2.05), those with low disease activity (DAS 28<3.2) had mean anti-CCP levels of 21.33 U/mL (SD=12.45), those with moderate disease activity (DAS 28 < 5.1) had increasing trend with mean anti-CCP level of 64.77 U/mL (SD=19.43) and the patients with high disease activity (DAS 28 > 5.1) were having the highest mean anti- CCP levels, 81.75 U/mL (SD=41.50).

DAS28 Score/No of patients	ANTI-CCP Expression (Mean ± SD)
Remission(4)	3.67 ± 2.05
Low (16)	21.33 ± 12.45
Moderate(86)	64.77 ± 19.43
High(44)	81.75 ± 41.50

Table 4: Anti-CCP antibodies titres in serum of patients with different activity disease level.

Discussion

Citrullinated proteins have recently been described as specific antigens of rheumatoid antibodies. In the recent years, serum anti-CCP positivity at baseline has been demonstrated to possess very high predictive and prognostic accuracy in comparison to other markers [16]. Its high specificity and its early presence in the disease, suggests that it plays an important role in the RA pathogenesis [17-19]. Anti-CCP antibodies have also demonstrated prognostic utility with regard to radiographic outcomes [20]. Interestingly, a recent study showed that early introduction of methotrexate therapy in undifferentiated arthritis patients with circulating anti-CCP delays evolution to RA, and prevents joint damage [21].

In this study we analysed the presence and levels of anti- CCP in 150 RA patients and 30 healthy controls to assess the diagnostic performance of anti-CCP test in RA patients. We also compared the levels of anti- CCP with various disease severity markers of RA patients. In our study the anti-CCP was positive in 78.66% of RA patients while no healthy control was positive for anti-CCP. The specificity and sensitivity of test was 100% and 78.67% respectively and the PPV was 100% and NPV was 48.39%. The area under the ROC curve was 0.88, thereby predicting the test to be having a good diagnostic accuracy. It should be noted that the value of any diagnostic test has been related to its disease prediction ability. Therefore our results support the role of anti-CCP as a good serological marker for RA.

In our study we found a significant association between the increased levels of anti-CCP and female RA patients as compared to male RA patients. We also observed a significant association between the increased anti-CCP levels and RF positivity in RA patients. Earlier studies have also reported a relation between anti-CCP and RF positivity in RA patients [22]. A greater prevalence and levels of anti-CCP in patients with higher DAS 28 and ESR values was also observed thereby indicating its role in more active disease. Furthermore, this observation was strengthened as a significant linear increase in the mean levels of anti-CCP was observed among increasing disease activity groups as measured by DAS28 Score. Many earlier studies have also observed greater prevalence and level of anti-CCP in patients with higher DAS 28 and CRP values, indicating its association with more severe disease, thereby supporting our results [23,24]. The greater prevalence of anti-CCP in patients with higher DAS 28 supports the hypothesis that this auto is associated with a more severe form of disease. Although we have not studied a relation between anti- CCP level and disease duration, but our data does suggests that patients with higher baseline anti-CCP levels have a more aggressive form of disease. This paper identifies the clinical value of anti-CCP tests in the diagnosis of RA supporting the validity of anti-CCP tests for this application. Given the promising results that anti-CCP is indicative of more severe disease, it would be beneficial for additional prospective studies with good patient follow up to further validate the prognostic value of anti-CCP tests.

Conclusion

In summary, we conclude that anti-CCP is a very good serological marker for RA diagnosis and a very important risk factor for more aggressive form of RA in our Kashmiri population. Therefore early detection may help clinicians in early diagnosis of RA that would result in appropriate and early management of the disease. As this is a cross-sectional study, we cannot reach to a cause effect conclusion. This is the main limitation of this study, but the association is strong enough to support the hypothesis that the anti-CCP positivity is a risk factor for poor prognosis in RA patients.

Conflict of interest

None declared

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References

1. Smolen JS (1996) Autoantibodies in rheumatoid arthritis. In: van Venrooij WJ, Maini RN, editor. Manual of biological markers of disease 34: 25-36.
2. Miriovsky BJ, Michaud K, Thiele GM, O'Dell JR, Cannon GW, et al. (2010) Anti-CCP antibody and rheumatoid factor concentrations predict greater disease activity in men with rheumatoid arthritis. Ann Rheum Dis 69: 1292-1297.
3. Vossenaar ER, Nijenhuis S, Helsen MM, van der Heijden A, Senshu T, et al. (2003) Citrullination of synovial proteins in murine models of rheumatoid arthritis. Arthritis Rheum 48: 2489-2500.
4. Masson-Bessière C, Sebbag M, Durieux JJ, Nogueira L, Vincent C, et al. (2000) In the rheumatoid pannus, anti-filaggrin autoantibodies are produced by local plasma cells and constitute a higher proportion of IgG than in synovial fluid and serum. Clin Exp Immunol 119: 544-552.
5. Masson-Bessiere C, Sebbag M, Durieux JJ, Girbal- Neuhauser E (2002) In the rheumatoid pannus, antifilaggrin autoantibodies are produced by local plasma cells and constitute a Deborah PM. Epidemiology of rheumatoid arthritis: Determinants of onset, persistence and outcome. Clin Rheu 111: 172-77.
6. Bartoloni E, Alunno A, Bistoni O, Bizzaro N, Migliorini P, et al. (2012) Diagnostic value of anti-mutated citrullinated vimentin in comparison to anti-cyclic citrullinated peptide and anti-viral citrullinated peptide 2 antibodies in rheumatoid arthritis: an Italian multicentric study and review of the literature. Autoimmun Rev 11: 815-820.
7. Nishimura K, Sugiyama D, Kogata Y, Tsuji G, Nakazawa T, et al. (2007) Meta-analysis: diagnostic accuracy of anti-cyclic citrullinated peptide antibody and rheumatoid factor for rheumatoid arthritis. Ann Intern Med 146: 797-808.
8. Bizzaro N, Tonutti E, Tozzoli R, Villalta D (2007) Analytical and diagnostic characteristics of 11 2nd- and 3rd-generation immunoenzymatic methods for the detection of antibodies to citrullinated proteins. Clin Chem 53: 1527-1533.

9. Taylor P, Gartemann J, Hsieh J, Creeden J (2011) A systematic review of serum biomarkers anti-cyclic citrullinated Peptide and rheumatoid factor as tests for rheumatoid arthritis. Autoimmune Dis 2011: 815038.
10. Bukhari M, Thomson W, Naseem H, Bunn D, Silman A, et al. (2007) The performance of anti-cyclic citrullinated peptide antibodies in predicting the severity of radiologic damage in inflammatory polyarthritis: results from the Norfolk Arthritis Register. Arthritis Rheum 56: 2929-2935.
11. RÄnnelid J, Wick MC, Lampa J, Lindblad S, Nordmark B, et al. (2005) Longitudinal analysis of citrullinated protein/peptide antibodies (anti-CP) during 5 year follow up in early rheumatoid arthritis: anti-CP status predicts worse disease activity and greater radiological progression. Ann Rheum Dis 64: 1744-1749.
12. Gerli R, Bartoloni Bocci E, Sherer Y, Vaudo G, Moscatelli S, et al. (2008) Association of anti-cyclic citrullinated peptide antibodies with subclinical atherosclerosis in patients with rheumatoid arthritis. Ann Rheum Dis 67: 724-725.
13. Rantapää-Dahlqvist S, de Jong BA, Berglin E, Hallmans G, Wadell G, et al. (2003) Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. Arthritis Rheum 48: 2741-2749.
14. Nielen MM, van Schaardenburg D, Reesink HW, van de Stadt RJ, van der Horst-Bruinsma IE, et al. (2004) Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. Arthritis Rheum 50: 380-386.
15. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, et al. (2010) 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum 62: 2569-2581.
16. van der Linden MP, van der Woude D, Ioan-Facsinay A, Levarht EW, Stoeken-Rijsbergen G, et al. (2009) Value of anti-modified citrullinated vimentin and third-generation anti-cyclic citrullinated peptide compared with second-generation anti-cyclic citrullinated peptide and rheumatoid factor in predicting disease outcome in undifferentiated arthritis and rheumatoid arthritis. Arthritis Rheum 60: 2232-2241.
17. Mierau R, Genth E (2006) Diagnosis and prognosis of early rheumatoid arthritis, with special emphasis on laboratory analysis. Clin Chem Lab Med 44: 138-143.
18. Curis E, Nicolis I, Moinard C, Osowska S, Zerrouk N, et al. (2005) Almost all about citrulline in mammals. Amino Acids 29: 177-205.
19. Vossenaar ER, van Venrooij WJ (2004) Citrullinated proteins: sparks that may ignite the fire in rheumatoid arthritis. Arthritis Res Ther 6: 107-111.
20. Meyer O, Labarre C, Dougados M, Goupille P, Cantagrel A, et al. (2003) Anticitrullinated protein/peptide antibody assays in early rheumatoid arthritis for predicting five year radiographic damage. Ann Rheum Dis 62: 120-126.
21. van Dongen H, van Aken J, Lard LR, Visser K, Ronday HK, et al. (2007) Efficacy of methotrexate treatment in patients with probable rheumatoid arthritis: a double-blind, randomized, placebo-controlled trial. Arthritis Rheum 56: 1424-1432.
22. Amo DVDN, Bosch RI, Manteca CF, Polo RG, Cortina EL (2006) Anti-cyclic citrullinated peptide in rheumatoid arthritis: Relation with disease aggressiveness. Clinical and Experimental Rheumatology 24: 281-286.
23. Vallbracht I, Rieber J, Oppermann M, Förger F, Siebert U, et al. (2004) Diagnostic and clinical value of anti-cyclic citrullinated peptide antibodies compared with rheumatoid factor isotypes in rheumatoid arthritis. Ann Rheum Dis 63: 1079-1084.
24. Lindqvist E, Eberhardt K, Bendtzen K, Heinegård D, Saxne T (2005) Prognostic laboratory markers of joint damage in rheumatoid arthritis. Ann Rheum Dis 64: 196-201.