



Comparative Evaluation of RF and Anti-CCP Tests for Rheumatoid Arthritis Diagnosis: Insights from a Pakistani Population

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ABSTRACT

Rheumatoid arthritis (RA) is an autoimmune inflammatory disease that principally affects joints. Various diagnostic techniques are in use to detect RA based on the presence of auto-antibodies in patients' blood like rheumatoid factor and anti-cyclic citrullinated peptides. We aimed to detect RF and anti-CCP antibodies for diagnostic evaluation of RA. RF was done by the latex agglutination method while anti-CCP test was performed on Abbot AxSYM system using micro-particle enzyme immunoassay method. The analysis showed that RF test is more prevalent than anti-CCP to determine whether an individual has RA or not. In the present study RA is also found to be associated with age of the patients. To conclude RA is an age-related disorder that can be detected earlier by the presence of RF autoantibodies as compared to anti-CCP. These findings can be helpful for the clinical diagnosis of RA.

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Authors' Contribution

MW conducted the research and drafted the manuscript. RB contributed to manuscript writing and revisions. MA supervised the research work and provided guidance.

Key words

Rheumatoid arthritis, RF, Anti-CCP

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic autoimmune disorder that primarily targets joints leading to their inflammation and destruction. Progressive damage to joints results in disability (Firestein, 2003). However, its impact extends beyond joints, affecting various tissues and organs such as ligaments, tendons, muscles, cartilage, and bone (Firestein, 2017; Turesson *et al.*, 2003). This complex ailment exerts its influence globally, afflicting approximately 1% of the world's population, with a prevalence of 0.14% to 0.55% among Pakistanis (Ghouri *et al.*, 2023). It demonstrates a gender bias, affecting females three times more frequently than males. Although the onset of RA can occur at any stage but mostly it affects people between 40 to 60 years of age (Alamanos and Drosos, 2005; Alamanos *et al.*, 2006). Moreover, the influence of this autoimmune disorder is heightened in smokers, with a

prevalence up to three times higher, especially among those who test positive for rheumatoid factor (Bang *et al.*, 2010).

RA is a mysterious ailment with origins still poorly understood. Genes, hormones, infections, and other autoimmune disorders are considered significant contributors (Firestein, 2017). While biological agents like bacteria, viruses and fungi are suspected culprits, we haven't proven it yet. Environmental factors such as tobacco use, exposure to silica minerals, and existing chronic conditions are prone to RA development (Klareskog *et al.*, 2006). The complex nature of RA involves inflammation driven by cytokines, specifically pro-inflammatory ones like leukotriene B4 (LTB4), tumor necrosis factor-alpha (TNF-alpha), interleukin-6, and interleukin-1b. These increased cytokine levels may lead to the deterioration of joints' synovium, synovial fluid, and cartilage, as revealed by serological studies (Brod, 2000). Genetic factors also play a substantial role, contributing to 50-60% of RA development (MacGregor *et al.*, 2000).

Patients with RA have a great variety of auto-antibodies in their serum. Detection of RA is based on these serological markers. The most commonly occurring auto-antibodies include rheumatoid factor (RF), cyclic citrullinated peptides (CCP), anti-perinuclear factor (APF), anti-keratin antibodies (AKA) and anti-filaggrin antibodies (AFA) (Nienhuis and Mandema, 1964; Young *et al.*, 1979; Hoet *et al.*, 1991; Schellekens *et al.*, 1998, 2000). RF is a classical antibody in RA diagnosis (Jaskowski *et al.*, 2010).

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According to American College of Rheumatology criteria for Rheumatoid Arthritis (RA), RF is the only reliable diagnostic antibody with high sensitivity (Avouac *et al.*, 2006). However, false negative results were also reported in many autoimmune diseases like chronic hepatitis, systemic lupus erythematosus (SLE) and Sjogren's syndrome (Dörner *et al.*, 2004; Sauerland *et al.*, 2005).

The Waaler-Rose hemagglutination assay is the prevailing method to analyze RF during the last 50 years (Plotz and Singer, 1956a, b). Many additional methods have also been developed, including different latex particle agglutination assays and isotype-specific enzyme immunoassays using various sources of IgG antibodies. The biological role of RF is not clearly understood. Therefore, various studies using different detection methods have contradictory results regarding sensitivity and specificity of RF test (Bas *et al.*, 2002).

Cyclic citrullinated peptides (CCP) are particular type of proteins. During inflammation arginine residues in proteins such as vimentin can be enzymatically converted into citrullinated peptides, in a process called citrullination. If their shapes are significantly changed, then these proteins may be taken as antigens by the immune system, provoking an immune response. The reactivity of RA sera depends on the citrulline residues present, since the same peptides in which the citrulline was replaced by any other amino acid were not antigenic (Raptopoulou *et al.*, 2007). These auto-antibodies can be detected even prior to development of clinical symptoms of joint destruction and inflammation. Recent studies have documented that anti-CCP antibodies showed good specificity in early detection of RA than any other class of antibodies (Rantapää-Dahlqvist *et al.*, 2003; Vallbracht *et al.*, 2004). Several assays for detecting anti-citrullinated peptide antibodies (ACPAs) were developed employing mutated citrullinated vimentin (MCV-assay), flaggrin-derived peptides (CCP-assay) and viral citrullinated peptides (Coenen *et al.*, 2007; Nishimura *et al.*, 2007).

When patients are positive for CCP as well as for RF, it is very likely that they may develop a severe form of the disease. When patients are negative for either CCP or RF, then the clinical symptoms are more important in determining the disease outcome. When patients are negative for both the CCP and RF, it is less likely that they may have RA (Feldmann *et al.*, 1996). Present study aims to clarify the comparative role of these auto-antibodies in diagnosis of RA in a local Pakistani population.

MATERIALS AND METHODS

Blood samples were collected from suspected RA patients from different Laboratories in Lahore for

diagnosis of RA from February 2022 to February 2023. Total number of samples collected was 65 out of which 29 were males of 12-74 years of age and 36 were females of 15-70 years of age. These samples were tested for RF and Anti-CCP.

For qualitative determination of RA in serum the latex agglutination method described by Plotz and Singer (1956a, b) was used. For the determination of Anti-CCP, diagnostic kit of ABBOT AxSYM was used which determined the IgG class of autoantibodies specific to cyclic citrullinated peptides in human serum and plasma (Schellekens *et al.*, 1998, 2000). The data was analyzed by using Chi-square test.

RESULTS

Table I describes overall prevalence of RF and anti-CCP in RA. Out of 65 samples for suspected RA patients, 41 were found positive for RA on the basis of RF, which constituted about 63% on the other hand 29 of this sampled population were positive for RA on the basis of anti-CCP with a prevalence of 44.6%. All anti-CCP positive patients were those that are RF-RA positive as well. Statistically significant difference was observed between two groups for the detection of RA (p -value <0.05).

Table I. Overall prevalence of RF and Anti-CCP in RA.

Total	RF +ve (%)	RF -ve (%)	Anti-CCP +ve (%)	Anti-CCP -ve (%)
65	41(63%)	24 (37%)	29 (44.6%)	36 (55.4)
p -value		0.03		
Chi-square value		4.45		
Df		1		

Table II. Gender wise prevalence of RF and anti-CCP in RA.

RF +ve		Anti-CCP+ve	
Male	Female	Male	Female
19	22	12	17
p -value 0.68			
Chi-square value 0.17			
Df 1			

Table II describes the gender wise prevalence of RF and anti-CCP in RA. Out of 29 males, 19 were positive for RF, which constituted 65.5%, while 22 females were positive for RF out of 36 which constituted 61.1%. The individuals positive for anti-CCP were 29, out of them 12 were male (41.3%) while 17 were females with a prevalence of 58.7%. No significant difference was

observed between males and females with reference to positivity for anti-CCP and RF (p -value >0.05).

Table III. Age wise prevalence of RF and anti-CCP in RA.

RF +ve		Anti-CCP +ve	
≤ 32 years	> 32 years	≤ 32 years	> 32 years
9	32	1	28
p -value 0.02			
Chi-square value 4.749			
Df 1			

Table III describes the age wise prevalence of RF and anti-CCP in RA. Among sampled population, 23 were in the age of 32 years or below while 42 were above 32 years of age. In the age group of 32 years or below, 9 were positive for RF with a prevalence of 39.1%. In the age group of above 32 years, 32 were positive for RF with a prevalence of 76.1%. In the age group of 32 years and below 3 were positive for anti-CCP (13%). On the other hand in the group above 32 years of age, 28 were positive for anti-CCP (61.9%). A significant difference (p -value < 0.05) was observed in two age groups for the prevalence of RF and anti-CCP in RA patients.

DISCUSSION

RA is disease of an unknown origin. Infections, genes, hormones and certain environmental factors such as smoking and exposure to heavy metals are considered to be associated with disease onset. It usually begins with a minor joint pain, stiffness and fatigue (Klareskog *et al.*, 2006; Linn-Rasker *et al.*, 2006). Being systemic illness, it also affects other organs, including skin, heart, blood vessels, lungs and liver etc. (Scott *et al.*, 2010).

In the present study, suspected RA patients were analyzed for RF and anti-CCP auto-antibodies. The prevalence of RF was found to be higher (63%) in patients, than that of anti-CCP. Among RF +ve RA cases it was observed that 70% were positive for anti-CCP. It reflected that RF is more sensitive than anti-CCP in RA patients. Similar findings are reported by Jaskowski *et al.* (2010). They observed that RF has a prevalence of 80% while anti-CCP has a prevalence of 65% among suspected RA patients. There were more anti-CCP +ve specimens among RF +ve subset of RA. The prevalence of anti-CCP positivity was 76% in RF +ve patients of RA. Scherbaum *et al.* (1984) observed the increased levels of RF while studying an association between RA and autoimmune thyroiditis (Scherbaum *et al.*, 1984).

Results of the present study for greater sensitivity of RA are supported by van Venrooij *et al.* (2002). They described that RF test has good sensitivity for RA but its specificity is low for the disease as it can also be detected in the serum of patients with other rheumatic or inflammatory diseases and even in the healthy population (van Venrooij *et al.*, 2002). However, during the present study RF sensitivity was studied in RA exclusively. Other autoimmune diseases were not considered that may have some confounding effects on presence of RF.

Contradictory results are also reported by various studies for comparative efficacy of RF and anti-CCP tests. van Gaalen *et al.* (2005) observed that although CCP-assays provide a sensitivity comparable with that of RF, however analysis of the correlation of anti-CCP antibody titer with RA disease activity yielded conflicting results (van Gaalen *et al.*, 2005). Gottenberg *et al.* (2005) observed in a comparative analysis of RF with anti-CCP that the proportion of rheumatoid factor (75.6%) and the proportion of anti-CCP positivity (75.9%) were significantly high (Gottenberg *et al.*, 2005).

Oliver and Silman (2006) reported that females are three times more affected by RA than males. RA is more common among females than males, which may be due to gender related hormonal differences (Oliver and Silman, 2006; Gibofsky, 2012). Alamanos and Drosos (2005) reported that females are three times more affected by RA than males (Alamanos and Drosos, 2005). During the present study gender wise prevalence of RA was also studied. More females were found positive for RF and anti-CCP than males. However, the difference was not significant statistically ($p > 0.05$) which might be due to short sample size in our study.

RA is considered an age-related disorder. The age wise prevalence of RA was also studied in RF +ve and anti-CCP +ve cases. In the age group of 32 years and below the prevalence of the disease was very low; on the other hand prevalence was significantly high in the group of above 32 years of age. These findings are in accordance with the findings of Alamanos and Drosos (2005) who reported that onset of the disease occurs more frequently between 40 to 60 years of age. Results of the present study are also similar to the findings of Scott *et al.* (2013) who reported the peak age of RA onset is above 40 years of life. This high prevalence may be related to age related hormonal changes, nutritional factors, long term exposure to heavy metals and oxidative stress as associated risk factors with the onset of RA in late stages of life.

CONCLUSION

From results of the present study, it is concluded that

RF is more efficient, sensitive and cost-effective strategy than anti-CCP for diagnosis of RA. Moreover, the onset of disease is also found to be associated with the age of the patients as well. However small sample size is a limitation of this study. Future studies involving large population fraction may be helpful in revealing greater details.

DECLARATIONS

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Ethical statement and IRB approval

All procedures were in agreement with the declaration of Helsinki. The Advance Study and Research Board at University of Education, Lahore approved the protocol of the present study. Permission from Ethical Committee, University of Education, Lahore, was also taken for the research work.

Consent for publication

All authors consented for the publication.

Availability of data and materials

Data and materials will be made available as required.

Informed consent

Informed consent was obtained from all individual participants included in the study.

Statement of conflict of interest

The authors have declared no conflict of interest.

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