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USE OF ANTI-CYCLIC CITRULLINATED PEPTIDE (ANTI-CCP) ANTIBODIES AND RHEUMATOID FACTOR (RF) IN DIAGNOSIS OF RHEUMATOID ARTHRITIS

Medicine

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ABSTRACT

INTRODUCTION: Rheumatoid arthritis (RA) is a chronic, systemic, inflammatory autoimmune disease that initially affects small joints, progressing to larger joints, and eventually the skin, eyes, heart, kidneys, and lungs. This study was conducted to determine the usefulness of anti-CCP antibodies and Rheumatoid Factor (RF) in diagnosis of Rheumatoid Arthritis and to estimate the diagnostic value of anti-CCP antibodies compared with Rheumatoid Factor (RF) in the diagnosis of Rheumatoid Arthritis.

MATERIALS AND METHODS: This study was conducted in the Department of Medicine and it was a cross sectional study. A total of 86 patients presented with clinically suspected rheumatoid arthritis who attended the OPD Medicine were consecutively recruited. The present study was conducted on patients with clinically suspected rheumatoid arthritis from Department of Medicine, Darbhanga Medical College & Hospital, Laheriasarai, Bihar over a period of 12 months.

RESULT: Anti-CCP antibody was not significantly associated with the disease activity (p value =0.36). Anti-CCP antibody was significantly associated with radiological defects whereby majority of patients with radiological defects (n=21/30; 70%) were positive for anti-CCP antibody (p value=0.03). Anti-CCP antibody was not significantly associated with the incidence of rheumatoid nodule (p value =0.750). Anti-CCP antibody was not significantly associated with extra-articular manifestation (p value =0.398). There is no significant association between anti-CCP antibody and incidence of pulmonary involvement (p value =0.367).

CONCLUSION: Combined use of RF and anti-CCP is a better prognostic and diagnostic tool than conventional RF tests alone. Uses of anti-CCP in clinical practice contribute to enhance the ability of rheumatologists to make judicious treatment decision. The usage of anti-CCP antibody is useful in the detection of early disease as evidenced by significant association between anti-CCP antibody, RF, and radiological involvement in our study.

KEYWORDS

Rheumatoid arthritis, anti-CCP, Rheumatoid Factor, radiological defects.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, systemic, inflammatory autoimmune disease that initially affects small joints, progressing to larger joints, and eventually the skin, eyes, heart, kidneys, and lungs. Often, the bone and cartilage of joints are destroyed, and tendons and ligaments weaken.[1] All this damage to the joints causes deformities and bone erosion, usually very painful for a patient.

The overall world prevalence of RA is approximately 0.5% to 1%.[2] There is regional variation in the prevalence of RA. The incidence appears to be highest in Pima Indians (5.3%) and Chippewa Indians (6.8%), and lowest in people from China and Japan (0.2%-0.3%), suggesting the possibility that genetic factors contribute to RA.[3] These differences in regional RA prevalence also may suggest a role for environmental factors.

Common symptoms of RA include morning stiffness of the affected joints for > 30 min, fatigue, fever, weight loss, joints that are tender, swollen and warm, and rheumatoid nodules under the skin. The onset of this disease is usually from the age of 35 to 60 years, with remission and exacerbation.[4] Clinically, the diagnosis of RA can be differentiated from osteoarthritis (OA) as the affected areas in RA are the proximal interphalangeal (PIP) and metacarpophalangeal (MP) joints; OA typically affects the distal interphalangeal (DIP) joint.[1] OA is the most common type of arthritis and is caused by wear and tear rather than an autoimmune condition. It has no effects on the lungs, heart, or immune system. In addition, OA typically affects only one side of the body, as opposed to the symmetrical nature of RA.[5]

The laboratory test which may contribute to the diagnosis of RA is that for rheumatoid factor.[6] However, the positive rate is approximately 70%. Since many rheumatic or immune diseases, including systemic lupus erythematosus (SLE), Sjogren's syndrome (SS), primary cryoglobulinemia, and viral infection or tumor may develop positive RF, the specificity of RF in RA is apparently lower.[7] Therefore, it is necessary to search for other laboratory diagnostic markers with high sensitivity and high specificity.

Since 1960, many investigators used indirect immunoflurorescence and enzyme-linked immunosorbent tests to detect serologic antibodies in RA patients.[8] These consisted of anti-perinuclear antibody (APF), anti-keratin antibody (AKA), anti-filaggrin antibody (AF), anti-Sa, anti-RA 33, and others. Although the specificity was higher (88%–99%) in some of those tests, the overall sensitivity (36%–59%) was lower and thereby limited their use as a routine laboratory test in RA.

Schelleken in 1988 reported that 76% of RA patients had a specific antibody which could interact with a synthetic peptide which contained the amino acid citrulline.[9] The arginine of the original substrate from APF or AKA can be converted through a PAD (peptide arginine deaminase) enzyme to “citrulline”, which can be easily detected by anti-CCP antibody (anti-cyclic citrullinated antibody).[10] This modification actually improves the specificity up to 98%. Sensitivity recently increased to nearly 80% after we used the 2nd generation anti-CCP enzyme-linked immunosorbent test (ELISA).[11]

In the past 5–6 years, many studies have focused on the value of the clinical application of anti-CCP antibody in rheumatoid arthritis and other rheumatic diseases.[12] The high specificity (98%) of anti-CCP in patients with RA can exclude other rheumatic or immune diseases in patients with positive anti-CCP. In addition, the anti-CCP antibody test may help us detect or recognize RA earlier.[13]

The present study was conducted on patients with clinically suspected rheumatoid arthritis from Department of Medicine, Darbhanga Medical College & Hospital, to determine the usefulness of anti-CCP antibodies and Rheumatoid Factor (RF) in diagnosis of Rheumatoid Arthritis.

AIMS AND OBJECTIVES

- To determine the usefulness of anti-CCP antibodies and Rheumatoid Factor (RF) in diagnosis of Rheumatoid Arthritis.
- To estimate the diagnostic value of anti-CCP antibodies compared with Rheumatoid Factor (RF) in the diagnosis of Rheumatoid Arthritis.

MATERIALS & METHODS

Type of Study: The Study was designed as a cross sectional study in Medicine Department of Darbhanga Medical College and Hospital, Bihar

Place of Study: Department of Medicine, Darbhanga Medical College and Hospital, Bihar

SOURCE OF DATA: The present study was conducted on patients with clinically suspected rheumatoid arthritis from Department of Medicine, Darbhanga Medical College & Hospital, Laheriasarai, Bihar over a period of 12 months.

SAMPLE SIZE:
Considering pooled sensitivity of anti-CCP antibody in diagnosis of Rheumatoid Arthritis as 67%, α-error of 5% and 10% precision, the estimated sample size was calculated as 86
Duration of Study: 24 months

INCLUSION CRITERIA:
- Clinically suspected rheumatoid arthritis patients.

EXCLUSION CRITERIA:
- Patients previously treated for Rheumatoid Arthritis.
- Other established cases of arthritis.

METHODOLOGY:
This study was conducted in the Department of Medicine and it was a cross sectional study. The study was conducted in a duration of 2 years. An informed consent was taken from all cases of clinically suspected rheumatoid arthritis patients and the necessary clinical details will be noted in the proforma prepared for the purpose. A total of 86 patients presented with clinically suspected rheumatoid arthritis who attended the OPD Medicine were consecutively recruited.

Demographic data was collected under the following headings: age, sex, anthropometric parameters. Weight and height were measured while patients were dressed in light clothing. Body mass index was calculated as weight (kg) divided by the square of the height (m).

About 5 ml of blood was acquired from all participants for anti-CCP and RF tests. Blood was collected after taking universal precautions. Serum was separated by centrifugation and was subjected to IgM-RF test, anti-CCP antibody detection by enzyme linked immune sorbent assay (ELISA), C-Reactive protein and ESR.

Anti-cyclic citrullinated peptide (CCP):
The detection of anti-CCP was performed by ELISA. Standard protocol was followed according to manufacturer's instruction. Results were reported qualitatively with reading of > 18U/ml was considered as positive.

Rheumatoid factor (RF):
In our laboratory, RF detection has been routinely tested by latex agglutination method as for the method mentioned with the kit. The result was reported qualitatively. Sera that agglutinate with the latex particle were considered as positive.

Table 1: Association of Anti-CCP antibody with Rheumatoid Factor

Rheumatoid Factor	Anti-CCP Positive (n=47)		Anti-CCP Negative (n=39)		Total	P Value
	Frequency	**Percentage**	**Frequency**	**Percentage**		
Positive (n=54)	36	41.9	18	20.9	54	0.003
Negative (n=32)	11	12.8	21	24.4	32	
Total	47		39		86	

Table 2: Association of Anti-CCP antibody with Disease Activity

Disease Activity	Anti-CCP Positive (n=47)		Anti-CCP Negative (n=39)		Total	p value
	Frequency	**Percentage**	**Frequency**	**Percentage**		
High	16	18.6	17	19.8	33	Chi Square-0.822 p value-0.36
Low	31	36.0	22	25.6	53	
Total	47		39		86	

Table 3: Association of Anti-CCP antibody with Radiological Defects

Radiological Defects	Anti-CCP Positive (n=47)		Anti-CCP Negative (n=39)		Total	p value
	Frequency	**Percentage**	**Frequency**	**Percentage**		
Positive	21	24.4	9	10.5	30	Chi Square-4.379 p value-0.03
Negative	26	30.2	30	34.9	56	
Total	47		39		86	

Table 4: Association of Anti-CCP antibody with Extra-articular Manifestations

Extra-articular Manifestations	Anti-CCP Positive (n=47)		Anti-CCP Negative (n=39)		Total	P Value
	Frequency	**Percentage**	**Frequency**	**Percentage**		
Present	16	18.6	10	11.6	26	Chi Square-0.713 p value-0.398
Absent	31	36.0	29	33.7	60	
Total	47		39		86	

STATISTICAL ANALYSIS: Data was checked for accuracy and completeness then coded and entered into (Statistical Package for the Social Sciences) version 19.0 for analysis. The results presented in frequency tables, cross tabulations and figures. Categorical data are presented as frequency with percentages. Continuous data with normal distribution are presented as mean with standard deviation. Chi-square test was the method used to test the significance of difference between two proportions. A p value <0.05 was considered statistically significant.

RESULT AND DISCUSSION
Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disease characterized by arthrosynovitis as the main pathology. Persistent recurrence of arthrosynovitis may damage the intra-articular cartilage and bones, causing articular dysfunction and eventually resulting in joint deformities or even disabilities.[14] Genetic and environmental factors favour for the development of the disease.

The etiology or precipitating factor of this disease is unknown; however interaction between both genetic and environmental factors is thought to contribute to its occurrence.[182] The condition affecting approximately 0.5% to 1% of the world population based on the geographical distribution and ethnically diverse population.[15] RA is a systemic disease that also involves numerous extra-articular organs such as lung, eye, vessels, blood cells, and others. HLA class II molecules particularly HLA-DRB1 alleles are known to be present in RA patients. Recently, *Proteus mirabilis* has been implicated in the aetiopathogenetic mechanism of RA. Combination of smoking and HLA-DRB1 shared epitope alleles (SE) was shown to increase the risk of developing RA. Early and aggressive intervention with established or new drugs can reduce the pathogenic process, improve the function of the joint, and increase the quality of life.[16]

Early diagnosis and treatment are important to avoid unwanted complications of the disease. Rheumatoid factor (RF) is used to help in the diagnosis of RA. In fact, according to previous criteria for diagnosis of RA provided by American College of Rheumatology in 1987, RF is the only laboratory criteria included. RF is sensitive but lack specificity. The sensitivity ranges between 25-95% while specificity ranges from 31-95%. High rates of false positive RF were found in other connective tissue diseases namely SLE and Sjogren's syndrome and in patients with chronic hepatitis.[17]

However, RF is not very specific for RA and can be detected in other rheumatic disorders, Infections and in healthy individuals. The introduction of anti-cyclic citrullinated peptide (anti-CCP) antibody assay as a second domain in the criteria is a significant advance in rheumatological field. Anti-CCP antibodies are principally autoantibodies directed against citrullinated proteins in the synovium of RA patients.[6]

The sensitivity of the assay is similar to RF but it has better specificity. The sensitivity ranges from 39-94% and the specificity ranges from 81-100%. Recently, the new criteria for diagnosis of RA have been introduced and anti- CCP together with RF is included in the criteria.[18]

As the current therapeutic strategies in RA recommend aggressive regimens early in the course of the disease, diagnostic tests with high specificity are desirable for choosing optimal treatment.[6]

Knowing that detection of anti-cyclic citrullinated peptide (CCP) antibody could improve the specificity of RA diagnosis. This study was intended to explore the usefulness of anti-CCP antibodies and Rheumatoid Factor (RF) in diagnosis of Rheumatoid Arthritis and to estimate the diagnostic value of anti-CCP antibodies compared with

Rheumatoid Factor (RF) in the diagnosis of Rheumatoid Arthritis.

Over the last decade, there has been particular interest in antibodies to citrullinated peptides and proteins as important etiological and predictive factors in early RA. Citrullination of proteins is a post-translational modification, which can occur as a normal part of cell apoptosis. However, this process may induce antibody formation in susceptible individuals, which may precede clinical arthritis by several years. Subsequent environmental triggers may enable anti- CCP antibodies to enter joints and contribute to a chronic inflammatory response.[19]

In this study, we found that the age of the patients with RA ranges from 16 to 60 years old with mean age of 36.47 years and female (80.2%) is the predominant gender.

Onset of RA at young age was seen in this study, which differed from other studies.[20,21] Women were more affected than men, which has been reported in one study.[6]

In this study, we assessed the association between the status of anti-CCP antibody (positive or negative) with various clinicodemographic and laboratory characteristics in a cohort of 86 RA patients. Majority of the patients were of middle age group at diagnosis with female predominance, consistent with other study showing similar age group distribution and female preponderance.[22]

It is widely acknowledged that bilateral joint pain or stiffness is among the most common complaint in rheumatoid arthritis patients, and majority will have pain involving multiple joint. In this study, most of the patients presented to the rheumatology clinic with bilateral pain or stiffness of small joints of the hands. According to the recently published classification criteria for rheumatoid arthritis, clinical synovitis is an important clinical presentation that should be looked into when evaluating rheumatoid arthritis patients.[23] If the patient is having clinical synovitis irrespective of duration that clinically can be assessed by presence of joint swelling then the individual will be tested further. For previous diagnosis criteria of rheumatoid arthritis which was based on American College of Rheumatology criteria 1987, clinical presentation of joint pain, swelling and morning stiffness must be present for at least six weeks before diagnosis of rheumatoid arthritis can be considered. Multiple and symmetrical joint pains are the common presenting complaint of patients with rheumatoid arthritis.[19]

In the study by **Gupta et al**., radiographs of hands and feet showed erosions in 50.8% of the patients.[24]

Anti-CCP$^+$ in RA patients varied among various studies. Seropositivity (anti-CCP) improved with generation of ELISA used. **Schellekens et al**. developed synthetic linear peptides based on filaggrin to be used as the antigen in serum RA tests.[25] Antibody recognition was further improved by the development of cyclic peptides rendering the citrulline moiety more available to serum antibodies; these tests were referred to as anti-cyclical citrullinated protein tests.[112,199] Second- and third-generation anti-CCP tests use combinations of synthetic cyclic citrullinated peptides selected through a screening process to increase sensitivity.[26]

For serological tests, both RF and anti-CCP antibody were positive in almost half of the patients with prevalence of41.9%, consistent with previous studies.[27] Our study demonstrated that RF was significantly associated with anti- CCP antibody positivity and this finding was supported by **Chou et al**[28] where RF was found to be significantly co-occurred with anti-CCP antibody in a cohort of 155 Chinese RA patients.

As reported by **Vencovsky et al**, radiological damage and early erosions were higher in combination with RF and anti-CCP antibodies.[29] Early detection of radiological involvement is pivotal to prevent irreversible complication and anti-CCP antibody has been shown to be predictive of radiological involvement. It is ideal if the disease progression can be monitored using ultrasound musculoskeletal in addition to DAS28-ESR score as it is easily performed.

Recent study showed that the seronegative RA is clearly has different genetic predisposition compared to seropositive group. In the latest Classification Criteria of Rheumatoid Arthritis 2010, both assays are included as laboratory markers to help diagnose the disease.[23]The latest guideline highlights the importance of quantitative value of the marker. A higher value will give higher score. With incorporation of these laboratory markers within the diagnostic criteria, early RA cases will be identified. This will prompt treatment to be initiated as early as possible, and ensures prevention of complication of intractable joint and bone damage.

CONCLUSION

At the end of the study, we come to the conclusion that:

Anti-CCP antibody serves as a better diagnostic marker in the diagnosis of RA. It supports the diagnosis of RA when RF is negative. Combined use of RF and anti-CCP is a better prognostic and diagnostic tool than conventional RF tests alone. Uses of anti-CCP in clinical practice contribute to enhance the ability of rheumatologists to make judicious treatment decision.

The usage of anti-CCP antibody is useful in the detection of early disease as evidenced by significant association between anti-CCP antibody, RF, and radiological involvement in our study.

Further study is required to determine underlying genetic association in patients with RA and the prognostic value of anti-CCP in RA.

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