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RF AND ANTI-CCP ANTIBODY TESTS- A COMPARATIVE STUDY IN THE DIAGNOSIS OF RHEUMATOID ARTHRITIS IN A TERTIARY CARE HOSPITAL

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ABSTRACT **INTRODUCTION:** Rheumatoid arthritis (RA) is autoimmune disease associated with chronic inflammation of joints causing deformities and functional impairment. Diagnosis primarily depends on clinical manifestations because of lack of suitable diagnostic tests. Rheumatoid factor (RF) is an autoantibody specific for Fc portion of human IgG. RF has low specificity as high false positive results are common in general population. Anti CCP antibody is also useful marker to diagnose rheumatoid arthritis and included in one of the criteria of American College of Rheumatology (ACR) /European League against Rheumatism (EULAR) classification of RA. Thus the present study was planned to compare the diagnostic utility of RF and Anti CCP antibody test in Rheumatoid arthritis patients in a Tertiary Care Hospital. **AIM & OBJECTIVE:** To compare the diagnostic utility of RF and Anti CCP antibody test in Rheumatoid arthritis patients. Material & **METHODS:** A total of 200 samples were taken from clinically suspected RA patients over a period of one year.RF was determined by latex agglutination method (SPAN DIAGNOSTICS Ltd.) and Anti CCP antibody by ECLIA (e-Cobas analyzer). The tests were performed as per manufacturer's instructions. **RESULTS & DISCUSSION:** Out of total 200 samples tested, Both RF and Anti CCP Antibody was positive in 100cases(50%). Only RF positivity was seen in 104(52%) and only Anti CCP antibody was positive in 140 (70%). We found Anti –CCP Sensitivity, Specificity,Positive predictive value,Negative predictive value result as 73.52% , 93.75%, 96.10%, 62.50% respectively.In present study combination of Anti CCP antibody and Rheumatoid factor together have shown positive predictive value for Rheumatoid Arthritis patients which lack specific signs and symptoms related to diagnosis of RA. Conclusion: Anti CCP antibody test and RF can be used concomitantly to diagnose Rheumatoid arthritis and can be used in clinical settings so that appropriate management can be initiated to decrease future morbidity.

KEYWORDS : Rheumatoid arthritis, Anti CCP antibody, Rheumatoid factor (RF).

INTRODUCTION

Rheumatoid arthritis is the most common inflammatory polyarthritis and is prevalent in approximately 1% population globally[1,2]. It is an autoimmune disease that occurs more commonly in women than men[3]. Approximately 20-30% of untreated rheumatoid arthritis patients become permanently disabled[4] . Thus, early and accurate diagnosis results in better treatment modality and lengthens healthy life. In 1998, Schelleken reported that antibodies, against citrullinated peptide, are highly specific seromarker for the diagnosis as well as prognosis of rheumatoid arthritis, before that rheumatoid factor (RF) was the only serological marker for the diagnosis of rheumatoid arthritis.[5,6]

The disease onset is usually gradually, with the predominant symptoms being pain, morning stiffness and swelling of many joints.[7] Early tends to affect smaller joints of hand and feet and later on as the disease progresses, symptoms often spread to the knees, ankles, elbows, hips and shoulders.[8,9] Rheumatoid arthritis is diagnosed according to clinical findings and serologic testing.

The main useful serological markers are rheumatoid factors and antibodies to citrullinated peptides. Rheumatoid factor (RF) is IgM autoantibody directed against the Fc portion of IgG. It is found in 75 to 80% of rheumatoid arthritis patients, but has a low specificity because it may be found in healthy elderly individuals and patients with other autoimmune diseases or infections.[10,11]

Anti cyclic Citrullinated peptide antibody (Anti-ccp) is autoantibody that bind antigenic determinant of unusual amino acid citrulline formed by posttranslational modification of arginine residues. The sensitivity of Anti-ccp for RA varies from about 50% to 75%, while specificity is relatively high, usually over 90%.[5,12].

The present study was undertaken to determine which serological marker is useful to diagnose rheumatoid arthritis and to compare cyclic citrullinated peptide antibody with rheumatoid factor.

MATERIALS & METHODS

This prospective study was carried out in Microbiology Department, Hind Institute of Medical Science,Sitapur India from JAN 2020 to DEC 2020.The study protocol was approved by the Ethical committee of the institution. The patients gave written informed consent to participate in the study. Blood Samples obtained in the Microbiolog Department were separated into sera and stored at - 20°C until for serology testing.

All laboratory works were undertaken in the Microbiology laboratory. Sera were subjected to serological tests like RF, Anti-CCP.

INCLUSION CRITERIA: A Total of 200 samples from clinically suspected RA patients over a period of one year from both genders were included in this study.

EXCLUSION CRITERIA: Patients of chronic renal failure, malignancy, diabetes mellitus, HIV, HBV and HCV, and pregnant women.

RF was determined by latex agglutination method (SPAN DIAGNOSTICS Ltd.). A Positive reaction is indicated by any observable agglutination in the reaction mixture. The specimen reaction should be compared to the RF Negative control.Recently, the identification of citrulline as a target of a whole set of autoantibodies like anti-perinuclear factor (APF), anti-keratin antibodies (AKA), anti-filaggrin antibodies (AFA) etc. detected in the sera of RA patients has led to the development of anti-CCP (Antibody to cyclic citrullinated peptide) assays that possess a high specificity for RA. The clinical performance of anti-CCP assays has been further improved by the use of multiple citrullinated peptides, resulting in a second generation of anti-CCP assays.

The Elecsys Anti-CCP assay uses a set of cyclic citrullinated peptides and is therefore a so-called second-generation assay.The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on Elecsys and cobas e 411 immunoassay analyzers. Immunoassay for the in vitro semi-quantitative determination of human IgG autoantibodies to cyclic citrullinated peptides in human serum and plasma.

Measuring range

7-500 U/mL (defined by the Limit of Blank and the maximum of the master curve). The Limit of Blank and Limit of Detection were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) requirements.

The tests were performed as per manufacturer's instructions.

Diagnostic characteristics of Anti-CCP tests were calculated in respect of sensitivity, specificity, Positive predictive value (PPV), Negative predictive value (NPV), by SPSS software 20.0.

RESULT :-

Total 200 patients comprising of 128(64%) females and 72(36%) males, shown in Figure 1.

After distributing the RF positive patients , gender –age wise, we found that in males ,highest percentage of 50% were found in 21-40 yrs of age groups, followed by 21.42% in 61-80 yrs , and similar result of 14.28% in two age groups (less than 20 years & in 41-60 yrs), with no case reported in > 80 years of age, as shown in table 1(a).

The ,Table 1 (a), also shows that in females ,highest percentage of 58.88% ,were found in 21-40 years of age groups, followed by 33.33 % in age group of 41-60 years, and 8.3% in < 20 years of age, with no case reported in age groups of 61-80 yrs, >80 years of age.

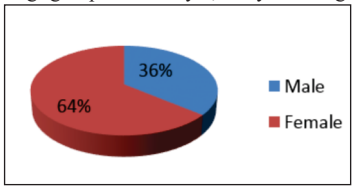

Figure 1: Gender distribution

Table 1(a); Gender-age wise distribution of RF positive Patients

Age groups	RF positive =104 (52%)	
	M=56 (53.84%)	F=48(46.15%)
<20 yrs	08 (14.28%)	04(8.3%)
21 - 40 yrs	28 (50%)	28(58.33%)
41 – 60 yrs	08 (14.28%)	16 (33.33%)
61 – 80 yrs	12(21.42%)	00 (0%)
>81 yrs	00 (0%)	00 (0%)

Table 1(b); Gender-age wise distribution of Anti- CCP positive Patients,

Age groups	Anti- CCP positive =140 (70%)	
	M=56 (53.84%)	F=48(46.15%)
<20 yrs	08 (12.5%)	24 (31.75%)
21 - 40 yrs	40 (62.5%)	28 (36.84%)
41 – 60 yrs	04(6.25%)	24 (31.57%)
61 – 80 yrs	12 (18.75%)	00 (0%)
>81 yrs	00 (0%)	00 (0%)

Gender-age wise distribution of Anti- CCP positive Patients,showed that in males, highest 62.50% is found in 21-40 years of age –group, 18.75% in 61-80 yrs , 12.5% in < 20 years of age group,followed by 6.25 % in 41-60 years & no case reported in >80 years of age.

While in females , highest percentage of 36.84% was found in 21-40 yrs of age group, similar result of 31.57% were observed in < 20 yrs, 41-60 yrs of age group, with no case reported in 61-80 yrs of age group and >80 yrs of age, as shown in Table 1 (b).

Out of 200 tested samples, 104 (52%) samples were positive only for RF, 140 (70%) were positive only for Anti – CCP antibody. Both RF & Anti – CCP antibody were found positive in 100 (50%) cases and 60 (30%) cases were found negative for both RF & Anti – CCP antibody which were shown in Figure ; 2.

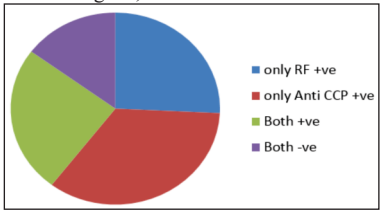

Figure ; 2.Distribution of sample results in percentage;

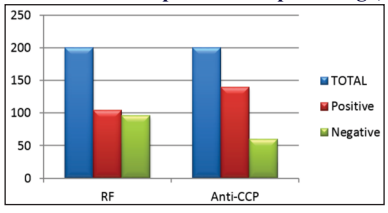

Figure 3 : Comparison of Positivity of RF and Anti-CCP antibody in clinically suspected RA cases .

Figure 3 shows, out of 200 tested samples, rheumatoid factor was found positive in 104 (52%) and negative in 96 (48%)samples. While Anti- CCP was found positive in 140(70%) and negative in 60 (30%) out of 200 clinically suspected samples.

Figure 4; Shows that , Out of 96 RF seronegative samples, 36 (37.5%) were found Anti – CCP antibody positive and 60 (62.5%) were Anti – CCP antibody negative.

In 104 RF seropositive samples, 100 (96%) were found Anti – CCP antibody positive and 4 samples (3.84%) showed no Anti –CCP antibodies.

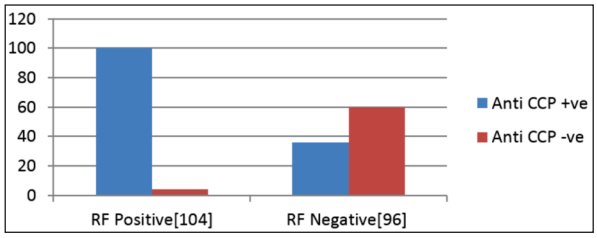

Figure 4; Comparison of Anti- CCP in RF positive and negative samples.

Table 2; Diagnostic Characteristics Of Anti-ccp Antibody Detection For The Diagnosis Of Ra

Anti- CCP			Sensitivity	Specificity	PPV	NPV
RF positive	Positive	negative	73.52%	93.75%	96.1%	62.5%
	100	04				
RF negative	36	60				

In our study, after considering Anti-CCP antibodies as a Standard test ,we found that ,out of 200 tested samples, 100 samples were positive for both Anti –CCP & RF{ True Positive(TP =100)},4 samples were found as Anti-CCP negative & RF positive {False Positive (FP=4)}; 36 samples were Anti-CCP positive & RF negative { False Negative(FN=36)},& 60 samples were both tests negative{True Negative(TN=60)}.

After applying the formula of Sensitivity =TP/(TP+FN), Specificity =TN/(TN+FP) , Positive Pedictive value (PPV)= TP/ (TP+FP), Negative Pedictive value (NPV)=TN/(TN+FN), we found Anti -CCP result as 73.52% , 93.75%, 96.10%, 62.50% respectively.

DISCUSSION :-

Anti-cyclic citrullinated peptide (anti-CCP) antibody testing is particularly useful in the diagnosis of rheumatoid arthritis, with high specificity, presence early in the disease process, and ability to identify patients who are likely to have severe disease and irreversible damage. However, its sensitivity is low, and a negative result does not exclude disease. Anti-CCP antibodies have not been found at a significant frequency in other diseases to date, and are more specific than rheumatoid factor for detecting rheumatoid arthritis.

We discuss anti-CCP antibody testing in rheumatoid arthritis, with an emphasis on diagnostic performance, prognostic capability, and relevance to pathogenesis and new treatment paradigms in rheumatoid arthritis.

The present study was conducted to facilitate the clinicians to confirm the diagnosis of rheumatoid arthritis. In the present study ,total 200 patients comprising of 128(64%) females and 72(36%) males. Majority of the patients (83.1%) were female that correlates with the studies conducted by Kiran Bala *et al* 2018[13] ;Sebbeg *et al.,* 1995[14]; Vincent *et al.,* 1989[15]; Nienhuis *et al.,* 1964[16]

Our present study showed percentage of patients positive for both RF and anti-CCP; percentage of patients negative for RF but positive for anti-CCP and percentage of patients positive for RF and negative for anti-CCP , 50%, 70%, 52% respectively. Study done by Bizzaro[17] showed 36 %,5%, 27%, Zeng[18] showed 38%,9%,21%, Lee[19] showed 57%,10%, 15%, Vallbracht[20] showed 52% 13% 15% respectively.

Suzuki[21] study reported only percentage of patients negative for RF but positive for anti-CCP , 69%.while Bas[22], De Rycke[23] studies did not work on these parameters.

Study	RF+ & CCP+	RF- & CCP+	RF+ & CCP-
Bizzaro[17]	36%	5%	27%
Bas[22]	NA	NA	NA
Zeng[18]	38%	9%	21%
Lee[19]	57%	10%	15%
Suzuki[21]	NA	69%	NA
Vallbracht[20]	52%	13%	15%
De Rycke[23]	NA	NA	NA
Our study	50%	70%	52%

Table 3;-rf, Rheumatoid Factor; Rf+ And Ccp+, Percentage Of Patients Positive For Both Rf And Anti-ccp; Rf- And Ccp+, Percentage Of Patients Negative For Rf But Positive For Anti-ccp; Rf+ And Ccp - Percentage Of Patients Positive For Rf And Negative For Anti-ccp,na, Not Applicable.

Table 4 - Sensitivity and Specificity of Anti-CCP antibodies for RA

Study	Anti-CCP sensitivity	Anti-CCP specificity
Bizzaro[17]	44%	97%
Bas[22]	56%	90%
Zeng[18]	47%	97%
Lee[19]	66%	90%
Suzuki[21]	89%	88%
Vallbracht[20]	64%	96%
De Rycke[23]	74%	99%
Our study	73.52%	93.75%

Several studies have examined the performance characteristics of anti-CCP antibodies in RA patients, sensitivity ranging from 44 % to 89%,while specificity ranging from 88% -99%, tabulated in table no.

Our study showed sensitivity & specificity 73.52% & 93.75% respectively, almost similar to study done by De Rycke[23] which showed sensitivity & specificity 74% & 99% respectively.

Present study showed sensitivity of 73.52%, correlates with finding of De Rycke[23] study, showing 74 % sensitivity.

While Suzuki[21] study reported little higher sensitivity which is 89%, and in contrast,lower sentivity , reported by several studies, like Vallbracht[20], Bizzaro[17], Bas[22], Zeng[18], Lee[19] 64%, 44%, 56%,47% ,66% respectively.

Our finding of specificity 93.75%, correlates with many studies ,like Bizzaro[17], Bas[22], Zeng[18], Lee[19], Vallbracht[20], De Rycke[23] which showed 97%,90%,97%,90%,96%,99% sensitivity respectively, while Suzuki[21] reported little lower sensitivity of 88%.

Other studies conducted by Munevver *et al.,* [24] (65%), Sibel *et al.,* [25](60%), Machold *et al.,*[26] (55%) and Nehir *et al.*[27], (44.8%) have documented less sensitivity of anti- CCP antibodies Munevver *et al.,* 2008[24]; Sibel *et al.,* 2004[25]; Machold *et al.,* 2007[26]; Nehir *et al.,* 2005[27]. This could be due to the method of selection of suspected cases.

Among specific autoantibodies for RA, anti-cyclic citrullinated peptides (CCP)

antibodies are used more often than others. They are very important for the diagnosis of the disease. It is well known that their specificity for RA is very high, although their sensitivity is similar to that of rheumatoid factor (RF). The anti-CCP antibodies have recently been set as a prognostic factor for RA,

because patients with high levels of these autoantiobodies have destructive and erosive forms of the disease.[28.29]

In addition, the presence of anti-CCP antibodies can be detected long before the onset of clinical manifestations (14 years) which suggests their role in the pathogenesis of RA. Therefore, the presence of anti-CCP antibodies in patients with early RA will influence the therapeutic choice and optimal time to start the treatment.[30,31,32,33]

CONCLUSION

Anti-CCP has become a "key" serologic marker in RA. It can be used (1) as a test for early diagnosis of RA; (2) for the differential diagnosis between RA and other rheumatic or immune diseases; (3) for prediction of prognosis; and (4) for evaluation of treatment outcome. However, this does not mean that anti-CCP can replace RF in diagnostic and prognostic testing for RA. We recommend that Anti CCP antibody test and RF should be used concomitantly to diagnose early RA and can be used in clinical settings so that appropriate management can be initiated to decrease future morbidity.

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