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Research Article

Diagnostic Performance of Anti-RA33 Antibodies in Comparison with Rheumatoid Factor (RF) Positive and Negative Patients in Coimbatore Districts of Tamil Nadu.

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Abstract:
Rheumatoid factor (RF), an immunological marker is the very first sero-marker used in the diagnosis of RA. An alternative to this scenario was seemed to have been aided by another marker, RA33 in identification of RA patients, who come under the category of sero-negativity RF tests. The main aim is to compare Rheumatoid Factor (RF) and Anti- RA33, to ensure which marker is more effective in diagnosing the rheumatoid arthritis patients. The cross sectional study was conducted for about three years from February 2020 – March 2023. A total of one hundred and fifty serum samples were collected from patients which includes 70 seropositive for RA and remaining 80 from seronegative RA patients. Among the 70 RA positive patients, 19 positive for anti-RA33 antibodies and the remaining 51 were negative. In case of 80 RA negative patients, 16 were positive and remaining 64 negative for the same. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were 54.29%, 55.65%, 27.14% and 80.0% respectively. Anti RA33 has proved to be an amazing biomarker for seropositive as well as seronegative patients. In the case of seronegative patients, where RF is negative or undetectable, Anti RA33 serves as an indeed essential marker. The study concludes that anti-RA33 antibodies could be a good marker but statistically couldn't be able to make a decision that it is a better marker than the RF

Keywords: Rheumatoid arthritis; Rheumatoid factor; Anti-RA33 antibodies; Immune marker; Rheumatology

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Introduction:
Rheumatoid Arthritis (RA) a disease of systemic autoimmune origin is said to be the most run into sickness, which affects about 1% of the population globally, with an unclear etiology, proceeding with severe chronic inflammation that further leads to

mortality and functional loss [1-2]. A premature diagnosis is very much essential for this infection, as it may aid to barricade the damages caused in the joints by rendering beforehand precautionary treatments [3]. Rheumatoid factor (RF), an immunological marker is the very first sero-marker used in the diagnosis of RA and is still its onboard. This immune-marker is an autoantibody which is directed by targeting the fragment of Immunoglobulin G molecule and there are many isotypes are available viz., IgA, IgG and IgM [4]. These diagnostic tests are more vital to survey the RA patients and their response to the treatment, as rheumatoid factor is the most of the times used to test for prognosis. Regardless this test is more sensitive to RA but ironically it is not a specific variable for RA [5]. Anti-cyclic citrullinated peptide (Anti-CCP) antibodies functions against the synthetic citrullinated peptides and they are more specific markers in comparison to RF [6-8]. Even though both of these markers are proven to be good for the detection of RA in patients, some still show the symptoms of the clinical condition but failing in the diagnostic results, both in RF and Anti- CCP [8]. An alternative to this scenario was seemed to have been aided by another marker, RA33 in identification of RA patients, who come under the category of sero-negativity by the other two tests [9-15]. This test could assist to accelerate the sensitivity of the laboratory tests for diagnosing this clinical condition.

About 33kDA antigen is an auto-antigen was identified in sera of RA patients which employed immunoblot from soluble nuclear HeLa cell extracts [16]. The hnRNP-A2 and its spliced variants B1 and B2 are the epitopes of RA33 and is said to be an auto antigen to RA patients [4]. Hence identification of anti RA33 auto antibodies has been employed recently in the field of rheumatology and anti-A2/hnRNP, commonly known as anti- RA33 is considered as a valuable diagnostic marker for RA [4]. There have been few studies in literature stating that, rheumatoid patients about 35% of them are said to produce anti RA33 but there are also cases where these auto antibodies are not found. They are said to be detected in about 1/3rd of RA patients and about 1% of the healthy population. The main advantage was found that anti RA33 autoantibodies could be detected in patients with RA who have negative RF and Anti CCP antibodies [17]. The main purpose of the study is to compare the tests between Rheumatoid Factor (RF) and Anti- RA33, to ensure that which marker is more effective in diagnosing the rheumatoid arthritis patients.

Materials and Methods:

Study Group:

The cross sectional study was conducted in a tertiary care hospital and the period of study was about three years from February 2020 – March 2023. A total of one hundred and fifty serum samples were collected from patients which includes 70 seropositive for RA and remaining 80 from seronegative RA patients. The pediatric age group, ante-natal women were excluded from the study.

Data Collection:

The patient's basic information was collected from the participant viz., age, gender, and ethnicity. The specific details like duration of the disease, onset of the disease, joints involved etc., were also collected from patients. The consent and patients history were collected by a face to face interview with patients and they were clearly educated about the study.

Sample collection & preparation:

After getting the consent from the patient's about 3ml of blood was collected in a clot activator tube. The serum was separated and stored separately at -20°C for antibody testing.

Antibody Detection:

Rheumatoid factor was performed by latex agglutination method (Lab-care Diagnostics, India). The results were first observed qualitatively and the samples showed agglutination within two-three minutes were considered as positive. The positive samples were subjected to semi-quantitation. Anti RA33 antibodies was detected by using a commercially available RA33 ELISA kit (IMTEC- RA33 Antibodies ELISA kit (Human, Wiesbaden, Germany)). This kit was IgG based antibody. While performing the tests, the samples were thawed and brought to room temperature and procedure was followed according to the kits instruction manual. Briefly, the use of recombinant RA33 is used to detect the presence of anti- RA33 in patient's serum which are further identified with the help of peroxidase tagged secondary antibodies. Once the substrate is added the chromogenecity of the reaction determines the avidity or concentration of the antibodies. Finally the stop solution is added and a change in color is observed from blue to yellow [18]. At the end, the optical density values (OD) were measured at 450nm to identify the anti RA33 antibodies in a patient. The results were interpreted as per the manufacturer's protocol, the samples with ≥25 IU/ml was considered as positive (cut-off value).

Results:

A total of about 150 samples were taken into the study with consisting of 70 RA positive patients with 25 male and 45 female with Mean ± SD age of the patients = 51.7 ± 13.4 with 95% Confidence Interval (51.14 ±3.15) and 80 RA negative patients with 40 male and 40 female with Mean ± SD age of the patients 41.5 ± 15.6 with 95% Confidence Interval (41.5696 ±3.445). The age of the RA positive patients ranges from 28 to 83 years and for negative patients about 18 to 75 years old. Among the 70 RA positive patients, 19 tested positive for anti-RA33 antibodies and the remaining 51 were negative. In case of 80 RA negative patients, 16 were positive and remaining 64 negative for the same. The sensitivity and specificity was calculated for anti-RA33 antibodies in comparison with RF test. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were 54.29%, 55.65%, 27.14% and 80.0% respectively. According to the study results, the accuracy of the test is about

55.33%. There was no statistical significance observed between RF positive and negative patients in comparison with Anti-RA33 (p=0.302). Table – 1 shows the details of clinical symptoms of RA positive and negative patients. Joint Pain +Swelling (p=0.0019) and Anemia (p=0.0207) were statistically significant between RA positive and negative patients. Remaining parameters like Joint Pain + Fever + Stiffness, fever, fever with malaise and stiffness in the morning was not statistically significant.

Discussion:

Rheumatoid arthritis is quite difficult to diagnose at the early stage of the disease. Anti RA33 has proved to be an amazing biomarker for seropositive as well as seronegative patients [10]. However, RF is predominantly sensitive and low cost when compared to anti-RA33 in diagnosing RA, yet in terms of specificity, it doesn't cope with the units put up by the other markers [8]. In the case of seronegative patients, where RF is negative or undetectable, Anti RA33 serves as an indeed essential marker [13, 15]. In the present study, we identified 16 RA seronegative patients who were positive for Anti RA33, in which it symbolizes the positive predictive value in the diagnosis of Rheumatic arthritis. Considering the point, RF is also many times detected in perfectly healthy individuals, but the only difference in rheumatoid patients is its increased production, which has typically led to a search for new immune markers to spot the clinical condition [14, 19-21]. Some studies have established a connection between RA and anti-RA33 antibodies, which gave conflicting results when compared to the present study [13, 15]. Due to this, we have discovered many new autoantibodies that ultimately serve as insights to detect this clinical condition very early than anticipated, enabling us to more possible laboratory tests [8]. A research study was conducted by Tomoum *et al*., on 34 arthritis patients in the juvenile category, the results obtained showed about 66.7% positivity for anti-RA33 and it was the only study to reveal the highest rate of positive index towards the juvenile population [22]. Hence, the present study showed quite moderate positivity 27.1% in 70 rheumatoid patients and 20% negative patients in the adult population. The prevalence of RA is similar in India and other developing countries. The prevalence rate was higher when compared to other countries viz., China, Indonesia, the Philippines, and rural Africa. Knowledge of RA is highly questionable among the rural Indian population. Two surveys were conducted from New Delhi and Coimbatore districts of Tamil Nadu on basic knowledge and awareness about RA among the Indian population. The response rate was higher among the participants who had knowledge of arthritis and Rheumatologists [23-24]. Suhail *et al*., demonstrated the overall sensitivity and specificity for the anti-RA33 antibody are 97.3% and 76.47% respectively [13]. In the present study, we have recorded sensitivity and specificity of 54.29% and 55.65% of anti-RA33 antibodies. It is quite moderate when compared to the Suhail *et al.,* [13]. Finally, the study from Saudi has not recommended anti-RA33 autoantibodies as an immunodiagnostic marker for the diagnosis of RA [25]. However, the meta-analysis done on anti-RA33 has revealed that its specificity is about 90% for diagnosis but the sensitivity is 33% [5]. Harman et al. showed a good correlation between the anti-RA33 antibody and the disease activity of the patients [15]. However, in the present study, there was no association between the anti-RA33 and the RF positive and negative patients. The current study shows that the anti-RA33 accuracy was said to be about 55% yet it was able to detect the condition in RA-negative patients. Though AntiRA33 is not detected in all RA-positive patients, it still gives us a positive predictive value and a limelight about the patient's prognosis in terms of the clinical condition. The study is highly limited to the information related to the radiological features and therapeutics of RA. The study concludes that anti-RA33 antibodies could be a good marker but statistically the results obtained through this study, couldn't be able to make a decision that it is a better marker than the Rheumatoid Factor. This immune marker is to be evaluated in a large population for the recommendation of RA in early diagnosis.

Conflict of Interest: None to declare by the authors

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Table – 1: Clinical symptoms of RA positive and negative patients (n=150)

Clinical Symptoms	RA positive patients (n=70)	RA negative Patients (n=80)	P value*
Joint Pain +Swelling	26	12	**0.0019**
Joint Pain + Fever + Stiffness	05	12	0.1961
Fever	13	20	0.3662
Fever + malaise	09	16	0.2744
Anemia	12	27	**0.0207**
Stiffness of joints in the morning	05	03	0.4738

*P value ≤0.05 considered as statistically significant