Comparative Study Of Rheumatoid Factor - Igm Autoantibody Testing By Latex Agglutination Nephelometry And Elisa In Patients With Rheumatoid Arthritis

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ABSTRACT

Objectives: To test Rheumatoid factor (RF) IgM autoantibody in Patients with Rheumatoid Arthritis by various methods like latex agglutination, Nephelometer and ELISA. Comparative analysis of the sensitivity and specificity of the tests performed.

Materials and Methods: The study was conducted for a period of six months from June 2018 to November 2018 in a tertiary care hospital in Chennai. 90 patients attending Rheumatology OPD or admitted in the ward with the diagnosis of Rheumatoid arthritis, satisfying the inclusion and exclusion criteria were taken up for the study.

Inclusion criteria: Clinically diagnosed Rheumatoid Arthritis patient as per revised ACR 1987 classification criteria. Duration of symptoms (1yr- early (RA) 1 Yr. (Established RA).

Exclusion Criteria: Those with systemic connective tissue diseases like SLE, Scleroderma, MCTD, Sjogren syndrome, those with chronic liver diseases, tuberculosis, subacute Bacterial endocarditis, Pregnancy, Lympho reticular malignancies are excluded for the study. Those with onset 16 years of age are also excluded. Under aseptic precautions about 3ml of blood was collected from each Patient. Rheumatoid factor (RF) IgM was tested for each patient by all three methods Latex agglutination, Nephelometry and ELISA.

Results: IgM rheumatoid factor (RF) was detected in the sera of 90 patients with Rheumatoid Arthritis. The percentage of positivity for Rheumatoid factor by Latex agglutination, ELISA and Nephelometry were 41,64&60 respectively. Sensitivity and Specificity of ELISA when compared to nephelometry were 63 & 33% followed by latex agglutination 41 &59%.

Conclusion: Though nephelometry is considered as gold standard, in this study ELISA was highly sensitive more even than nephelometry in Rheumatoid factor detection followed by latex agglutination.

Keywords: Rheumatoid factor, Latex agglutination, Nephelometry, ELISA

1. INTRODUCTION

Rheumatoid Arthritis is a chronic inflammatory autoimmune disease characterised by articular involvement, synovial membrane inflammation, tissue infiltration by leucocytes and joint destruction. It affects mainly small joints of the hands and feet and has many systemic manifestations.

Rheumatoid factors (RFS) are autoantibodies directed against the FC fragment of human immunoglobulin IgG, present in majority of patients with Rheumatoid Arthritis. Rheumatoid

factors (RFs) are the first and the most common auto antibodies described in Rheumatoid arthritis (RA). RFs are also present in several other auto immune disease, infections as well as in healthy subjects. Measurement of RF is important in the diagnosis of Rheumatoid Arthritis and in determining prognosis. Patients with high titers of RF tend to develop extraarticular complications as well as increased severity of RA such as erosion, rapid disease progression and worse outcome. Rheumatoid factors are usually IgM, but can be IgG, IgA, IgE but not IgD, RFs are commonly detected by semiquantitative latex agglutination and standard quantitative methods like ELISA and nephelometry. This study compares all the three methods to determine the highly sensitive and the best method.

2. MATERIALS AND METHODS

The study was conducted in a tertiary care hospital for a period of six months from June 2018 to November 2018.

Inclusion Criteria:

Clinically diagnosed Rheumatoid Arthritis patient as per revised ACR 1987classification criteria. Duration of symptoms (1yr- early (RA) 1 Yr. (Established RA)

Exclusion Criteria

Those with systemic connective tissue diseases like SLE, Scleroderma, MCTD, Sjogren syndrome, Those with Chronic liver diseases, Tuberculosis, Subacute Bacterial endocarditis, Pregnancy, Lympho reticular malignancies are excluded for the study. Those with onset 16 years of age are also excluded.

Processing methods

Under strict aseptic precautions about 3ml of blood is collected from patients. Serum is separated and tested for Rheumatoid factor IgM by all the three methods.

*Latex Agglutination***:**

This test is done by using AVITEX RF Kit. Principle: Avitex RF Latex particles are coated with specially purified human gamma globulin as described by singer etal. When the latex suspension is mixed with serum containing elevated RF levels on a slide clear agglutination is seen within 2 minutes. The kit had a detection limit of 8 IU/ml of RF in the patient serum and the reagent is calibrated against the WHO international reference population.

Nephelometry

This test is carried out using NEPHSTAR. Rheumatoid Factor (RF) Kit Golsite diagnostics It is a protein analysis system for quantitative determination of human Rheumatoid factor. The Principle of particle enhanced immunonephelometric is applied. This method involves measuring the light scattered by insoluble complexes formed by reaction between specific protein in samples and its respective antibody covalently coupled to latex particles and the amount of scattered light is directly proportional to the concentration of the protein under condition that antibody is in excess. The sensitivity limit is 20 IU/ml

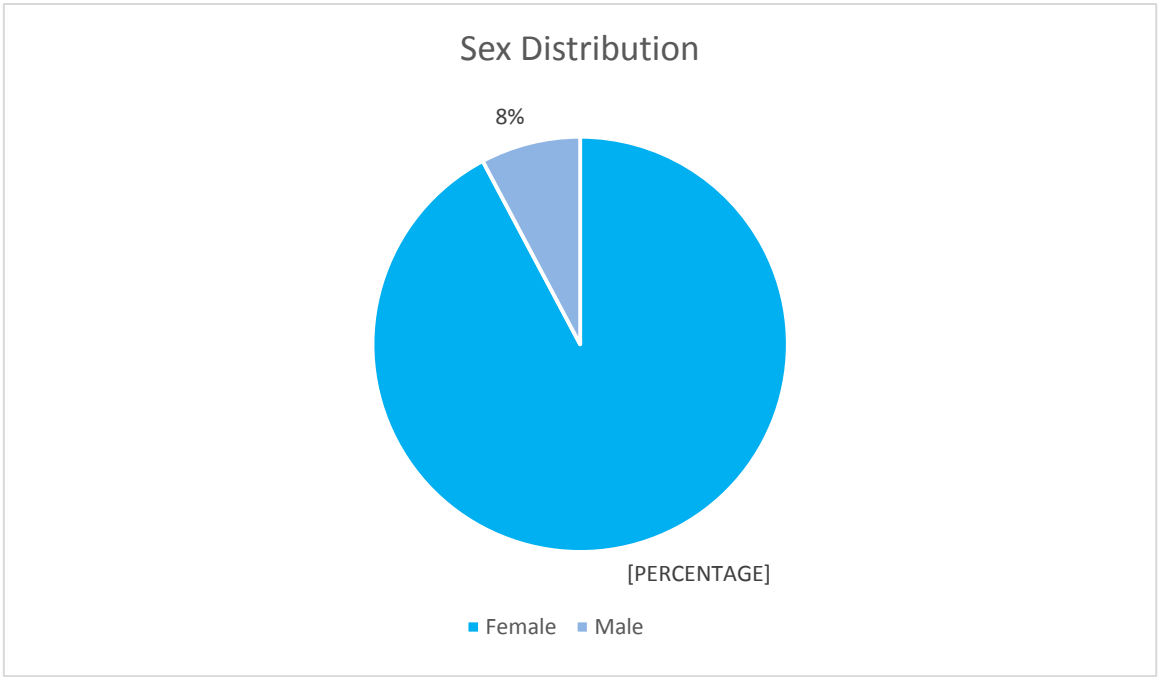
ELISA

This test is done by using EUROIMMUNE IgM Rheumatoid factor Elisa Kit.

Principle of the test. The test Kit contains microtiter strips each with 8 break off reagent wells coated with IgG. In the first reaction step diluted patient samples are incubated in the wells. In the case of positive samples, specific IgM antibodies (also IgA and IgG) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using peroxidase labelled anti-human IgM (goat) catalysing a colour reaction. Calibration is performed in relative units (RU) 20 Ru/ml is considered as positive for Rheumatoid factor IgM. <20RU/ml is negative for IgM Rheumatoid factor

3. RESULTS: -

Age and Sex distribution

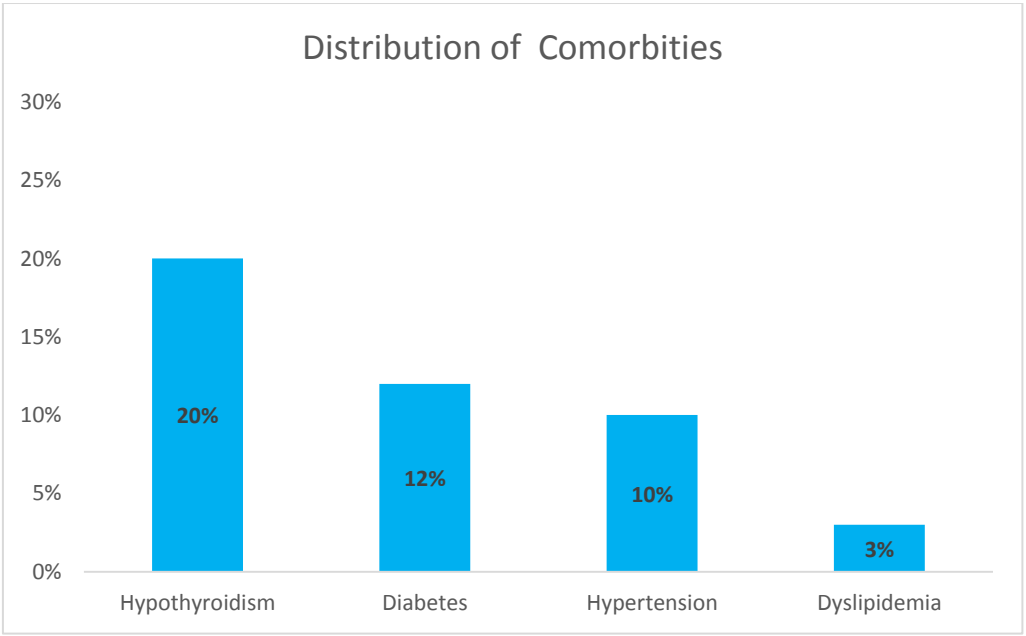


The most commonly affected age group is 16-40 yrs. The mean age affected were (42.01+_23.1 yrs.). Females were more commonly affected than males. Out of total 90 samples 92%were females and 8% were male patients.

Duration of Illness

Out of 90 People studied, 57 patients had 1 – 5 years duration of illness 17 patients had duration of less than 1 years, 14 patients had duration of 5-10 years. Only 2 patients had duration of illness of more than 10 years. the mean duration of illness in the study group is 3 yrs.

Comorbidities

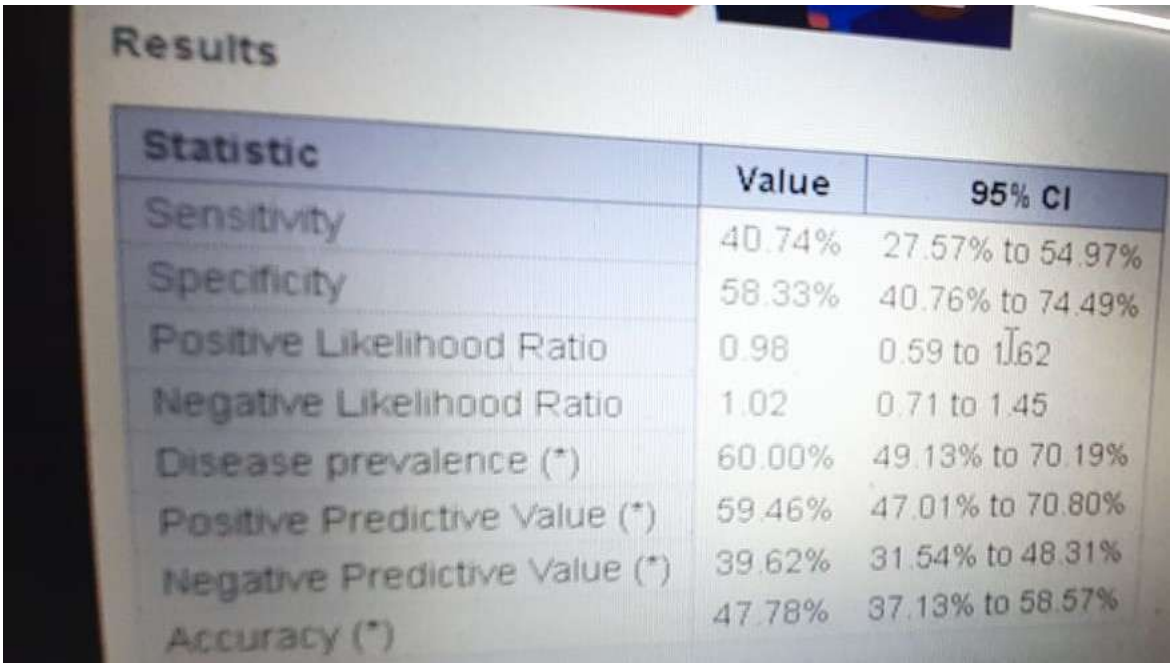


Out of 90 patients, 26% had comorbidities whereas 74% were without any comorbidities. Among the comorbidities 12% had diabetes,10% had hypertension.3% had dyslipidemia,20% had hypothyroidism. Hypothyroidism was seen more common in patients with Rheumatoid Arthritis.

Analysis of Rheumatoid factor testing by three methods:

Latex agglutination method:

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	NEGATIVE	53	58.9	58.9	58.9
	POSITIVE	37	41.1	41.1	100.0
	Total	90	100.0	100.0	

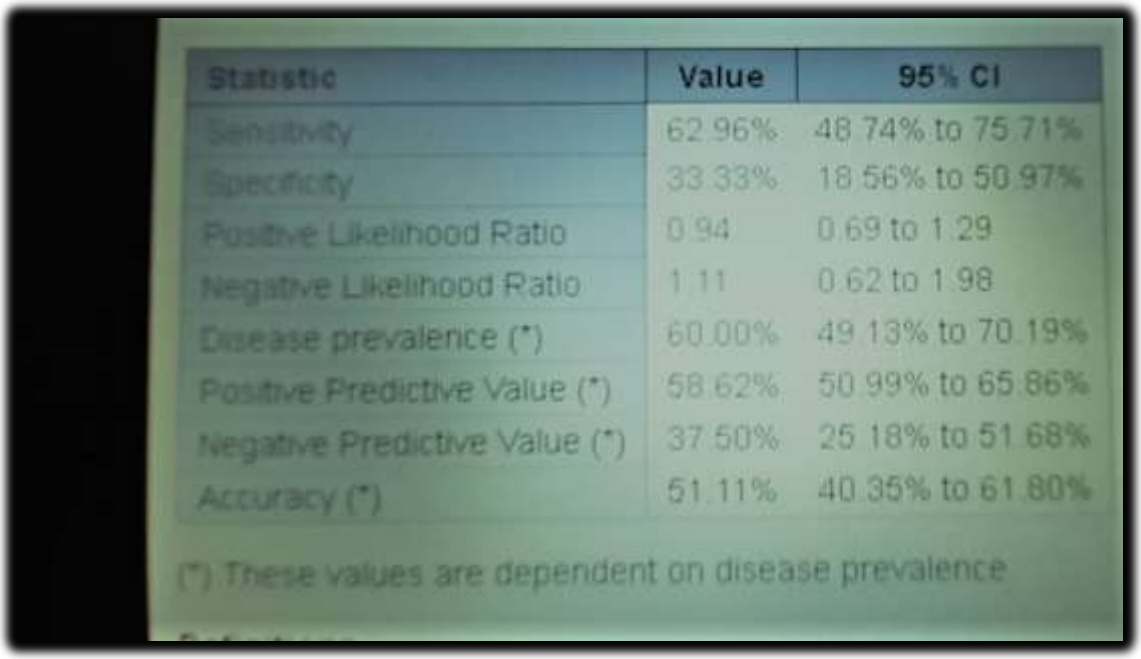

Results

Statistic	Value	95% CI
Sensitivity	40.74%	27.57% to 54.97%
Specificity	58.33%	40.76% to 74.49%
Positive Likelihood Ratio	0.98	0.59 to 1.62
Negative Likelihood Ratio	1.02	0.71 to 1.45
Disease prevalence (*)	60.00%	49.13% to 70.19%
Positive Predictive Value (*)	59.46%	47.01% to 70.80%
Negative Predictive Value (*)	39.62%	31.54% to 48.31%
Accuracy (*)	47.78%	37.13% to 58.57%

Out of 90 sera tested,37(41.1%) showed positivity for Rheumatoid factor by latex agglutination method. Sensitivity was 40.74%. Specificity was 58.33% positive predictive value is 59.46%. Negative predictive value is 39.62%.

ELISA:

ELISA					
Valid		Frequency	Percent	Valid Percent	Cumulative Percent
	NEGATIVE	32	35.6	35.6	35.6
	POSITIVE	58	64.4	64.4	100.0
Total		90	100.0	100.0	



Statistic	Value	95% CI
Sensitivity	62.96%	48.74% to 75.71%
Specificity	33.33%	18.56% to 50.97%
Positive Likelihood Ratio	0.94	0.69 to 1.29
Negative Likelihood Ratio	1.11	0.62 to 1.98
Disease prevalence (*)	60.00%	49.13% to 70.19%
Positive Predictive Value (*)	58.62%	50.99% to 65.86%
Negative Predictive Value (*)	37.50%	25.18% to 51.68%
Accuracy (*)	51.11%	40.35% to 61.80%

(*) These values are dependent on disease prevalence

In this method 58 (64%) were positive for Rheumatoid factor (RF). Sensitivity was 62.96%. Specificity was 33.33%. positive predictive value was 58.62%. Negative predictive value was 37.50%

Nephelometry

NEPHELOMETRY

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	NEGATIVE	36	40.0	40.0	40.0
	POSITIVE	54	60.0	60.0	100.0
	Total	90	100.0	100.0	

FREQUENCIES VARIABLES=ELISA
/ORDER=ANALYSIS.

In this method 54 (60%) showed positivity for RF

RF LATEX * NEPHELOMETRY Crosstabulation

		Nephelometry		Total
		Positive	Negative	
RF latex	Positive	22	15	53
	Negative	32	21	37
Total		54	36	90

ELISA * NEPHELOMETRY Crosstabulation

		Nephelometry		Total
		Positive	Negative	
ELISA	Positive	34	24	32
	Negative	20	12	58
Total		54	36	90

4. DISCUSSION:

Rheumatoid Arthritis is a systemic autoimmune disease characterized by the presence of autoantibodies and autoreactive T cells in peripheral blood and synovial fluid. Rheumatoid factor (RF) is the autoantibody detected in earliest stage of RA.

Rheumatoid factor (RF) is present in low titers in about 10 to 15 % of healthy elderly individuals.it is positive not only in RA, also in several other rheumatological & non rheumatological conditions. The prevalence of RF among diseased & healthy individuals varies according to the sensitivity and specificity of the method employed.

It was first identified in 1937 by agglutination of sheep red blood cells sensitized with rabbit IgG. Now several modifications of agglutination reaction are done. They are bentonite flocculation test& Latex agglutination test in which human and rabbit IgG were coated with substances such as latex particles & bentonite. Latex agglutination tests were shown to be most specific but less sensitive in the detection of RF. (1,2,3). This study also shows latex agglutination as most specific.

IgM RF is the main isotype detected by agglutination tests and it is not possible to measure either IgG RF or IgA RF. Recent studies shows that both IgM RF and IgA RF are also raised in seropositive RA and they are associated with more severe complications of the disease such as vasculitis, (4). Increased levels of IgA RF are also associated with erosive articular symptoms. (5,6,7).

In revised 1987 ACR criteria in diagnosis of RA positive RF test has been included. (8). Hence it is very essential to give accurate results that are comparable between laboratories.

ELISA is a suitable method that can be used routinely in clinical laboratories to detect and quantitate all isotypes of RF easily. They represent quick and cost-effective way to process large number of samples using simple and relatively inexpensive equipment. (9,10). This study IgM ELISA showed high sensitivity and specificity. Mota et al (11) the detection of isotype IgM as a useful marker to distinguish patients with polyarthritis that progressed to characteristic RA clinical status.

Viser et al (12) clearly showed that the ELISA test may be considered as a substitute to agglutination for detection of RF IgM.

RF detection by any of the three methods showed high seropositivity among females than males. Though the exact ethology is not known. Hormones play a vital role in development of disease in females (13)

5. CONCLUSION

This study showed ELISA had high sensitivity in detection of RF followed by Nephelometry followed by Latex agglutination. Though nephelometry is considered as gold standard, it requires expensive equipment's and kits. ELISA when compared to nephelometry is easy to perform, requires inexpensive equipment, used to test large numbers of samples at a time and is easy to carry out in any clinical lab.

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