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A COMPARATIVE DIAGNOSTIC EVALUATION OF RHEUMATOID FACTOR BY DIFFERENT IMMUNOLOGICAL TECHNIQUES: LATEX AGGLUTINATION, GELATIN AGGLUTINATION, TURBIDOMETRY AND ELISA IN RHEUMATOID ARTHRITIS

PRAMOD KUMAR VERMA*, USHA**, NILADRI***, NAND KUMAR SINGH**** AND SHYAM KUMAR SARAF*****

Declaration

The Declaration of the authors for publication of Research Paper in The Indian Journal of Research Anvikshiki ISSN 0973-9777 Bi-monthly International Journal of all Research: We, *Pramod Kumar Verma, Usha, Niladri, Nand Kumar Singh and Shyam Kumar Saraf* the authors of the research paper entitled A COMPARATIVE DIAGNOSTIC EVALUATION OF RHEUMATOID FACTOR BY DIFFERENT IMMUNOLOGICAL TECHNIQUES: LATEX AGGLUTINATION, GELATIN AGGLUTINATION, TURBIDOMETRY AND ELISA IN RHEUMATOID ARTHRITIS declare that , We take the responsibility of the content and material of our paper as We ourself have written it and also have read the manuscript of our paper carefully. Also, We hereby give our consent to publish our paper in Anvikshiki journal , This research paper is our original work and no part of it or it's similar version is published or has been sent for publication anywhere else.We authorise the Editorial Board of the Journal to modify and edit the manuscript. We also give our consent to the Editor of Anvikshiki Journal to own the copyright of our research paper.

Abstract

Rheumatoid Arthritis is an autoimmune disease with multifactorial triggering effects such as environment, infections and genetic influences. It causes polyarthritis of symmetrical joints especially the small joints of peripheral extremities (wrists, ankles and phalanges). Rheumatoid factor is a gold standard marker for the diagnostic utilization along with the ACR criteria. RF can be detected by different immunological techniques.

Diagnostic capability of different techniques for RF detection in Rheumatoid arthritis has been studied to look at the best screening test for diagnosis. The latex agglutination and other tests such as gelatin agglutination, turbidometry and enzyme linked immunosorbant assays were taken to study their diagnostic utility.

A total of 112 RA cases selected according to the ACR criteria were included in the study. Latex agglutination and other tests such as gelatin agglutination, turbidometry and enzyme linked immunosorbant assays (IgM, IgA and IgG) were done.

Among all the techniques, the ELISA was found to be the best diagnostic marker as it detects the positive values in 69.6% of RA cases while the gelatin agglutination has also good capability to trace the RF positivity (64.3%) followed by turbidometry method which was positive for RF in 58.9% of RA case. The latex agglutination method was not proved to be having an effective evaluation quality as it was positive in only 34.8% cases of RA.

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Although the ELISA for the RF isotypes (IgM, IgA and IgG), if done collectively, is the best technique for diagnosis of RA. Gelatin agglutination and turbidometry are the next to claim good diagnostic capability while the latex agglutination is a qualitative and bad marker.

In conclusion the screening technique for RF in Rheumatoid Arthritis should be either ELISA, gelatine agglutination or turbidometry. Gelatine agglutination is the most convenient method since it does not need any instrument and is sensitive, quantitative, cost effective and easy to evaluate.

Keywords: Rheumatoid arthritis, RF, latex agglutination, ELISA, gelatin agglutination, turbidometry.

Introduction

Rheumatoid arthritis is a polyarthritis of autoimmune etiology. It is affecting approximately 1% of human population world-wide and is characterized by considerable morbidity and mortality.[1] The most commonly used diagnostic markers for the rheumatoid arthritis are the auto-antibodies produced in response to auto-antigen such as IgG Fc and citrullinated fibrinogen. The quantitative values of auto-antibodies are used to represent the disease severity.

Rheumatoid factor is an antibody directed against the Fc portion of the immunoglobulin G (IgG) and is taken as one of the most important diagnostic tool approved by revised American College of Rheumatology Criteria, 1987[2] and Rheumatoid Arthritis Classification Criteria, 2010[3]**.** Many other auto-antibodies have been introduced but RF is still claimed to be the gold standard test[4]. Recently, senior rheumatologist's panel concluded that the higher value of serum RF level is still proved to be the best laboratory indicator of disease severity[5].

An indirect hem-agglutination test i.e. Waller-Rose assay in which the RBC sensitized with rabbit IgG was agglutinated by RF antibodies, was supposed to be the first test introduced for the RF detection**.** To eliminate the non-specificity provided by the RBC as carrier in passive agglutination, a modified gelatin particles were introduced as carrier which were sensitized with denatured rabbit IgG[6]. Among several other techniques used latex agglutination is another qualitative passive agglutination test. The quantitative methods such as turbidometry and Enzyme linked immunosorbant assays (ELISA) have also been added as the diagnostic markers. ELISA for the RF isotypes (IgM, IgA and IgG) is one the most widely used methods for the quantitative estimation.

Material and methods

A total of 114 Rheumatoid Arthritis cases have been taken in study. Cases were taken from indoor and outdoor of Department of Medicine and Orthopedics. Clinical details, radiological findings were recorded. Patients with known infection were excluded. The diagnosis was done according the Revised Criteria of American College of Rheumatology, 1987. Informed consent was taken from all the patients and the work was approved by the Institute ethical committee of this University.

RF was estimated by four methods for their comparative study in RA patients. RF by Latex agglutination, Gelatin agglutination, Turbidometry and RF isotype by ELISA were also done in the same cases of RA. RF by Gelatin agglutination was done by titration with the kit of Serodia and latex agglutination was done by kit of Tulip Company. RF isotypes (IgM/IgA/IgG) antibody were done by kit of DeMediTec, Germany. The latex agglutination was done qualitatively. The RF by the gelatin agglutination was taken positive if titre was >1:40. For ELISA of RF isotypes (IgM/IgA/IgG) value >60 IU/ml were taken as positive.

Results

Among all the techniques for RF detection, ELISA was the most sensitive test and it detects the RF positivity in 69.6% of RA cases when all the isotypes of RF antibody (IgM, IgA and IgG) were done together. The Gelatin agglutination shows RF positivity in 64.3% of RA cases while 58.9% were positive by the turbidometric technique. The Latex agglutination was the least sensitive as it was positive in only 34.8% of rheumatoid arthritis cases. (Table I)

A comparative analysis shows a very good concordance between ELISA and Gelatin agglutination. Gelatin agglutination showed 75.6% positivity in ELISA positive cases and was the most significant combination (p value = 0.000). The turbidometry method also have agreement with the ELISA up to 66.7% for RF detection in RA and it was also a significant combination (p value = 0.012). The latex agglutination was not good enough as it was positive in only 38.5% of ELISA positive cases (p value = 0.221;Table II)

To understand the agreement between ELISA and gelatin agglutination, different isotypes of RF alone and in combination have also been compared with ELISA. The positivity of RF IgM, RF IgA and RF IgG alone were non-significantly associated with gelatin agglutination positivity. RF IgA + IgG combination was also a non-significant association for concordance with gelatin agglutination. The combination of IgM + IgG and IgM + IgA were significant in relation to gelatin agglutination positivity as they were positive for gelatin agglutination in 100% of combinations with the p values 0.014 and 0.029 respectively. The results showed that gelatin agglutination was also positive in 93.0% of RA cases where all the three RF isotypes (IgM+IgA and IgG) were positive collectively which was a good combination and significant (p value = 0.000) as well. While in the cases positive for any RF isoypes alone and all the combination gelatin agglutination was positive in 77.8% cases (significant, p value = 0.000).

Discussion

Rheumatoid arthritis is an autoimmune disease in which many autoantibodies are produced. The RF positivity reported ranges from 50% to 80% of RA cases[7,8,9] . Our study shows 69.6% RF positivity by ELISA test and it was the most sensitive test among all the studied four techniques. In our study, gelatin agglutination is positive in 64.3% cases while the turbidometry (a test similar the nephalometry) shows 58.9% positivity. Similar to our study gelatine agglutination was reported slightly better than nephalometry[6]. ELISA should be preferred over latex agglutination test as report by Visser et al 1996[10]. The comparitive study among the RF positive by ELISA shows gelatine agglutination to be very much associated (75.6%) technique. The turbidometry was the next sensitive test with 66.7% RF positivity. Latex agglutination was the least sensitive and was positive in only 34.5% of these cases. Our study shows ELISA was the best technique and gelatine agglutination showed good association with it (p value = 0.000) followed by turbidometry which was also significantly associated with ELISA (p value = 0.012). Latex agglutination was the least sensitive and not associated to ELISA. However one study has reported latex agglutination and nephalometry is good enough to detect RF (p value = 0.000)[11]

The RA cases in which all the RF isotypes were positive gelatin agglutination shows very good sensitivity i.e. upto 93% ($\div^2$ = 16.940; p value = 0.000). The RF ELISA positive cases in alone RF isotypes and combinations show sensitivity 77.8% with p value of 0.000 proves the gelatine agglutination is also a very good quantitative method for RF detection. The Latex agglutination test is economical in comparison to Gelatin agglutination , Turbidometry and ELISA for RF (IgM/IgG/IgA). Now a days,

another autoantibody ACPA (anticyclic citrullinated antibody) is also claimed to be a very sensitive test and even recommended by the new American College of Rheumatology and European league against rheumatism, 2010.[12]

Conclusion

The present study reveals ELISA for RF isotypes (IgM, IgA and IgG) when taken together for the diagnosis is the best quantitative method. This test is most significantly fulfilling the need for the screening of RA with the quality result as quantitative. Gelatin agglutination test is easy to perform without any instrument or kit or effort. Turbidometry and latex agglutination test on the other hand are good but do not qualify the sensitivity level for a good screening test.

T A B L E I *RF detection by the various techniques in RA patients.*

Techniques	RF Positive		RF Negative		Total No. of RA Cases
	No.	%	No.	%	
Gelatin Agglutination	72	64.3	40	35.7	
ELISA (IgM/IgA/IgG)	78	69.6*	34	30.4	112
Latex Agglutination	39	34.8	73	65.2	
Turbidometry	66	58.9	46	41.1	

* Highest positivity

T A B L E II *Correlation of RF detection from ELISA to Gelatin Agglutination, Latex Agglutination and Turbidometric techniques.*

Group	RF Positive by Gelatin Agglutination		RF Positive by Latex Agglutination		RF Positive by Turbidometry	
	No.	%	No.	%	No.	%
RF ELISA Positive (78)	59	75.6	30	38.5	52	66.7
RF ELISA Negative (34)	13	38.2	9	25.5	14	41.2
Total No. of RA Cases	72	64.3	39	34.8	66	58.9
$\div^2$	14.430		1.500		6.357	
p value	0.000*		0.221		0.012*	

*Significant (p value > 0.05 was taken as significant)

T A B L E III *Correlation of RF detection form ELISA (RF isotypes; alone and in combination) to Gelatin Agglutination technique.*

RF isotypes Positive	Gelatin Agglutination RF Positive		Gelatin Agglutination RF Negative		$\div^2$	p value
	No.	%	No.	%		
IgM alone (2)	2	100.0	0	0.0	1.131	0.287
IgA alone (2)	0	0.0	2	100.0	3.665	0.056
IgG alone (17)	9	52.9	8	47.1	1.123	0.289
IgM + IgG (10)	10	100.0	0	0.0	6.100	0.014*
IgM + IgA (8)	8	100.0	0	0.0	4.786	0.029*
IgA + IgG (10)	4	40.0	6	60.0	2.821	0.093
IgM + IgA + IgG (32)	30	93.0	2	6.2	16.940	0.000*
Total RF alone and combinations	63	77.8	18	22.2	16.336	0.000*

*Significant

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