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Comparative Measurement of Rheumatoid Factor in Serum and Synovial Fluid of Rheumatoid Arthritis Patients by ELISA and Latex-Agglutination Test

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ABSTRACT

Rheumatoid factors (RF) are autoantibodies with specificity for the Fc portion of human IgG. Production of RF is a characteristic feature of rheumatoid arthritis (RA) and is detectable in high titer in about 90% of these patients. In this study, we measured total IgM, IgA, IgMRF and IgARF in serum and synovial fluid of 45 RA patients by ELISA and the results were compared with those obtained by latex agglutination test (LAT). The results show that from 45 RA patients, 93% (43:45) and 78% (35:45) were seropositive in ELISA and LAT, respectively. The sensitivity limit of LAT was approximately 10 µg/ml, whereas less than 10 ng/ml of RF was detectable by ELISA. There was a highly significant correlation between the concentration of IgMRF and latex agglutination titer in both serum (r = 0.78, p<0.0005) and synovial fluid (r = 0.66, p<0.05). Less significant correlations were observed for IgARF in serum (r = 0.65, p<0.05). While the concentration of total IgM and IgA were significantly higher in serum compared to synovial fluid, this however, was not reflected in the IgMRF or IgARF titer. In summary, our findings suggest that employment of isotype specific ELISA is inevitable and necessary for the quantitative measurement of RF isotypes other than IgM, which may be of clinical importance for diagnosis of disease severity and extra-articular complications of RA. *Iran. Biomed. J. 4 (2 & 3): 63-76, 2000*

Keywords: Rheumatoid factor, Rheumatoid arthritis, ELISA, Latex-agglutination test

INTRODUCTION

Production of high titers of rheumatoid factor (RF) in serum and synovial fluid is a characteristic feature of rheumatoid arthritis (RA) and may be implicated in pathogenesis of this disease [1-4]. Rheumatoid factors are autoantibodies with specificity for the Fc portion of human IgG and may be produced in multiple isotypes [5-7]. Deposition of RF-IgG complexes in the synovium and other tissues of RA patients has been taken as evidence to support their contribution in manifestation of the articular and extra-articular symptoms, frequently observed in these patients [2, 8, 9]. Rheumatoid factors may also serve physiological functions [1, 10] and produced in other inflammatory or lympho-proliferative disorders, though quantitatively and qualitatively different from those produced in RA patients [11-13].

Routine laboratory tests (Rose-Waaler test or Latex-agglutination test) which are employed for measurement of RF detect only multimeric RF, particularly IgMRF by their ability to agglutinate sheep red blood cells (SRBC) or latex particles coated with IgG [14-16]. Latex-agglutination test (LAT) is a qualitative method and can not detect other classes of RF, which may be of clinical value.

Quantitative measurement of different isotypes of RF has been performed by enzyme-linked immuno-sorbent assay (ELISA) and shown to be superior to the traditional LAT technique [17-19].

In this study, IgMRF and IgARF have been quantitated in serum and synovial fluid of Iranian patients with RA and the results were compared with those obtained by the traditional LAT.

MATERIALS AND METHODS

***Patients and clinical samples*.** Sera and synovial fluids were collected from 45 RA patients (17 males

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and 28 females, aged 47 ± 15 years), attending the Rheumatology clinic of the University Hospital of Hazrat Rasool in Tehran. The disease was diagnosed according to the American Rheumatism Association (ARA) criteria for classical RA [20].

Quantitation of total IgM and IgA. Total IgM and IgA were measured in serum and synovial fluid by a capture ELISA method, essentially as described previously [17, 21]. Briefly, polystyrene microtiter ELISA plates (Maxisorp, Nunc, Denmark) were sensitized with 10 µg/ml of purified monoclonal anti-IgM (AF6) or anti-IgA (2D7) antibodies (Kindly provided by Prof. R. Jefferis from the Univ. of Birmingham, UK). Following 90-min incubation at 37°C, the plates were washed three times with PBS containing 0.05% Tween 20 (Sigma, Chemical Co., UK) (PBS/T) and incubated with appropriate dilutions of the samples in PBS/T. Serial concentrations of affinity purified IgM (Kok) or IgA (A3) paraproteins were used to construct the standard curves. Bound IgM or IgA was revealed using appropriate dilution of F(ab)2 fragments of horseradish-peroxidase (HRP) conjugated sheep anti-human µ or α chains, respectively (Sigma). The plates were finally developed with O-phenylenediamine (OPD) (Sigma) substrate and optical density (OD) values measured at 492 nm using a Multiscan ELISA reader (Organon Teknika, The Netherlands). The concentration of IgM or IgA in each sample was determined by extrapolation from the corresponding standard curve.

Quantitation of IgMRF and IgARF. The ELISA method employed for measurement of IgMRF and IgARF has been described in detail elsewhere [21]. Briefly, ELISA plates were coated with 20 µg/ml of DEAE-cellulose purified human IgG. After 90 min incubation at 37°C, the plates were washed three times with PBS/T and incubated with appropriate dilutions of the samples in duplicate. Finally, isotype specific HRP conjugates were added and the plates were developed with OPD. The levels of IgMRF or IgARF were extrapolated from standard curves using the OD values developed for known inputs of affinity purified IgMRF paraprotein (Fr) or culture supernatants from IgARF producing hybridoma (B27) [22], (kindly provided by Dr. Mierau from the Rheumaklinik Aachen, Germany).

Qualitative detection of RF by LAT. RF was detected in serum and synovial fluid by LAT, using a commercial kit (Iran Pajohan, Iran). All the samples were tested at a dilution of 1:20 according to the manufacturer instruction. Positive results were assigned +1 to +4 based on strength of agglutination.

RESULTS

Quantitation of total IgM and IgA. Concentrations of total IgM and IgA were measured in serum and synovial fluid of 45 RA patients. The standard curves employed for extrapolation of IgM and IgA titers are illustrated in Figure 1.

Serum levels of IgM and IgA were significantly higher than those of the synovial fluid ($p<0.0001$ and $p<0.001$, respectively) (Table 1).

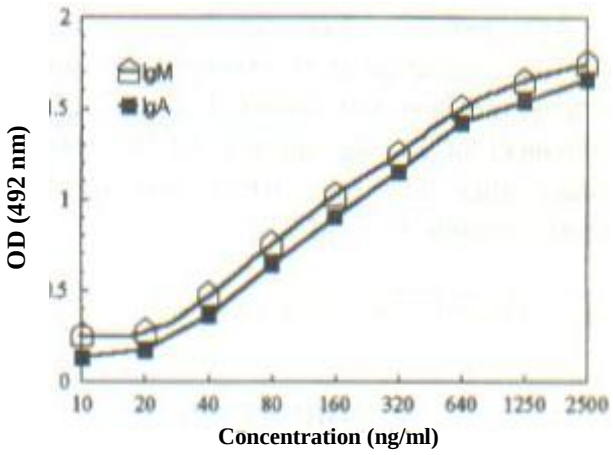

Fig. 1. Standard curves for measurement of total IgM and IgA by ELISA.

Quantitation of IgMRF and IgARF. IgMRF and IgARF were measured in serum and synovial fluid by extrapolation from the corresponding standard curves (Fig.2). Down to 10 ng/ml of RF was detectable by this ELISA method. From the RA patients, 93% (43:45) were positive for both IgMRF and IgARF (>1 µg/ml). There was no significant difference between IgMRF and IgARF in serum or synovial fluid (Table 1).

No significant correlation was found between the concentration of IgMRF and IgARF in either serum or synovial fluid. However, highly significant correlations were observed between the serum and synovial fluid levels of both IgMRF ($r = 0.8$, $p<0.002$) and IgARF ($r = 0.84$, $p<0.0001$).

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Table 1. Quantitation of IgM, IgA, IgMRF and IgARF levels in serum and synovial fluid of RA patients by ELISA.

Ig	Serum	Synovial Fluid	*P* value
IgMRF (µg/ml)	102 (112)[a]	98(120)	NS[b]
IgARF (µg/ml)	62.2 (76)	64(71)	NS
IgM (mg/ml)	2.31(1.1)	1.48(1)	<0.0001
IgA (mg/ml)	0.99 (.52)	0.64(.45)	<0.001

[a]SD: Standard Deviation, [b] N S: not significant;

Qualitative measurement of RF by LAT. Seventy eight percent (34:45) of the sera and synovial fluids agglutinated the latex particles. The titer of agglutination was similarly represented in both serum and synovial fluid (Fig. 3).

Comparison between LAT and ELISA methods. The results obtained by ELISA were compared with those of Latex method (Fig. 3). There was a highly significant correlation between the concentration of IgMRF and latex agglutination titer in both serum ($r = 0.78$, $p<0.0005$) and synovial fluid ($r = 0.66$, $p<0.05$). This correlation was less evident for IgARF in serum, but not synovial fluid samples ($r = 0.65$, $p<0.05$ and $r = 0.69$, $p<0.02$, respectively). Measurement of RF is important for the diagnosis of RA and in determining prognosis especially in high titer patients, as these patients tend to develop extra-articular complications. In addition, it has been proposed that RF may be important in pathogenesis of the disease [1-3].

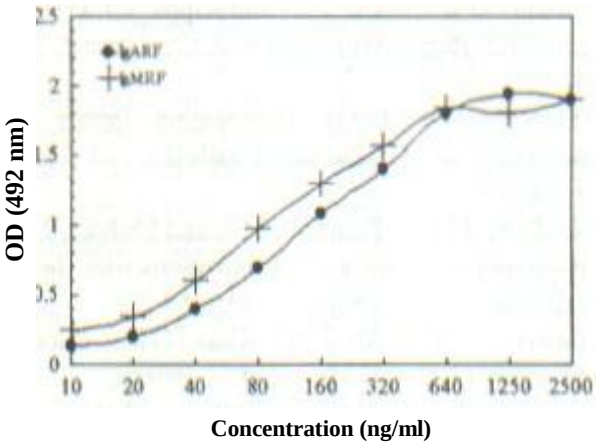

Fig. 2. Standard curves for quantitation of IgMRF and IgARF by ELISA.

DISCUSSION

RF was first identified in 1937 by agglutination of SRBC sensitized with rabbit IgG [16]. In the many years that followed a variety of clinical tests for RF were developed on the basis of agglutination reaction that used modifications of the original sheep cell test. In addition to SRBC, human and rabbit IgG were coated to substances such as latex particles and bentonite. The sensitized sheep cell agglutination test, the bentonite flocculation test and latex fixation test were shown to be the most specific in determination of RF, but the least sensitive [14, 15, 23]. These methods are also difficult to quantitate and there is a large variation in titers for the same sera between different laboratories. Furthermore, IgMRF is the main isotype detected by agglutination tests and it is not possible to measure either IgGRF or IgARF. Recent reports have suggested that both IgMRF and IgARF are also raised in seropositive RA, and may be associated with the more sever extra-articular complications of the disease such as vasculitis [24]. Increased levels of IgARF in RA patients have been found to be associated with erosive articular symptoms [6, 7, 11].

All isotypes of RF could be easily detected and quantitated by ELISA. More recently suitable ELISA methods have been reported which can be routinely used in clinical laboratories. These assays represent a quick and cost-effective way to processing large numbers of samples, while using simple and relatively inexpensive equipment [19, 25, 26]. However, despite their clinical potential, the ELISA methods have had a restricted routine usage and the classical agglutination assays are still performed in the majority of hospital service laboratories. If the ELISA assay is to gain general acceptance, it must carefully be compared with other methods.

In the present study, we have developed a sensitive ELISA for measurement of IgARF and IgMRF and the results were compared with those of LAT. A highly significant correlation was found between the two assays concerning the IgMRF levels ($p<0.0005$). Similar correlation, but to a lesser extent, was also observed for the IgARF titers ($p<0.05$) (Fig. 3).

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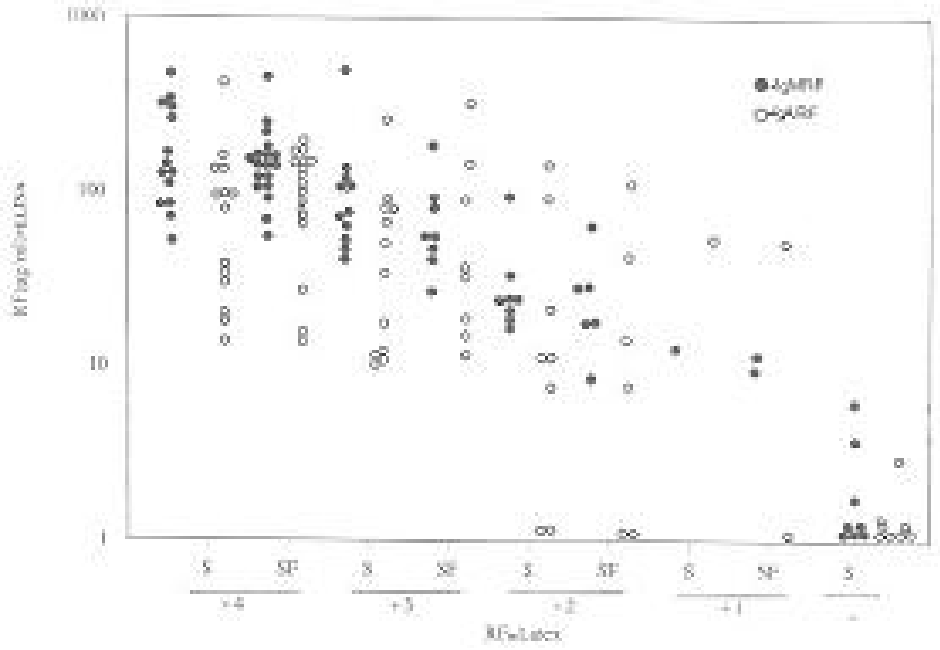

Fig. 3. Correlation between the results obtained from Latex-agglutination and ELISA methods for measurement of RF (S, serum; SF, synovial fluid)

These findings were not unexpected, since agglutination reactions are faciliated by multimeric antibodies. Therefore, pentameric IgMRF is more efficient than the dimeric IgARF in agglutinating the IgG coated latex particles. This explains the correlation observed for IgMRF between the two assays as compared to IgARF [17, 18]. The levels of total IgM and IgA were measured in both serum and synovial fluid to find out whether the titers of RF in both tissues are directly associated to the total immunoglobulin (Ig) levels. As outlined in Table 1, the results clearly indicate active production of RF in synovial fluid. Our findings also demonstrated a higher total IgM concentration as compared to IgA in both tissues. Although the serum level of IgA is expected to be higher in normal individuals, however, in systemic autoimmune diseases, including RA, higher concentrations of IgM have already been reported, due to polyclonal B-cell activation [4].

Assay sensitivity has also been investigated and compared in this study. While the sensitivity limit of LAT was approximately 10 µg/ml, down to 10 ng/ml of RF was detectable by ELISA. Since at least a dilution of 1:100 of serum or synovial fluid was tested in ELISA to minimize non-specific binding and improve assay specificity, therefore, samples having more than 1 µg/ml of RF gave positive results and were considered seropositive. Accordingly, a larger proportion of the RA serum samples tested could be assigned seropositive by ELISA as compared to LAT.

In summary, our findings suggest that employment of isotype specific ELISA is inevitable and necessary for the quantitative measurement of RF isotypes other than IgM, which may be of clinical importance for diagnosis of disease severity and extra-articular complications of RA.

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