

## EVALUATION OF ANTI-DS DNA ANTIBODIES IN ANTI-NUCLEAR ANTIBODY POSITIVE OMANI PATIENTS

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### ABSTRACT

**Objective:** To study the correlation between enzyme linked immunosorbent assay (ELISA) and immunofluorescent (IF) anti-dsDNA antibody measurement in different diseases.

**Patients:** One hundred and forty sera from patients with systemic lupus erythematosus (SLE, n=40), rheumatoid arthritis (RA, n=30), disease control (n=40) and from healthy control subjects (n=30) were included.

**Results:** Using the ELISA, serum anti-dsDNA was detected in 24/40 (60%) SLE, 5/30 (16.6%) RA, 9/40 (22.5%) disease controls and 1/30 (3.3%) of normal healthy controls. When IF assay was employed, anti-dsDNA antibodies were detected in 16/40 (40%) SLE, 3/30 (10%) RA, 5/40 (12.5%) disease controls but none in the normal subjects.

**Conclusion:** These results suggest that both ELISA and IF techniques can be used for the measurement of anti-dsDNA antibodies in clinical laboratories and, these antibodies are not unique to SLE.

**KEYWORDS:** Anti-dsDNA antibodies, ELISA, Crithidia luciliae, Systemic lupus erythematosus, Rheumatoid arthritis.

### INTRODUCTION

A general feature of systemic rheumatic diseases is the presence of circulating antibodies to a variety of cellular antigens. The detection of these antibodies plays a key role in the differential diagnosis and pathogenesis of autoimmune diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and mixed connective tissue disease (MCTD).<sup>1-4</sup>

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Anti-dsDNA antibodies constitute a heterogeneous family with respect to immunoglobulin class, avidity, complement-fixing ability, antigenic specificity and cross-reactive pattern.

Antibodies reacting to dsDNA mainly recognize the deoxyribose phosphate backbone; therefore, they are reactive to both single and double stranded forms of DNA. On the other hand, antibodies defined as reactive to single stranded DNA (ssDNA) recognize polymers of purine and pyrimidine bases which are available for reaction in the single stranded form of DNA but are not available in the dsDNA (native) form of DNA. Anti-ssDNA more commonly seen than those of dsDNA and often occur in the absence of dsDNA; however, they are nonspecific.<sup>5</sup>

The reported prevalence of circulating antibodies to dsDNA in SLE varies from 60%<sup>6</sup> to 80%.<sup>7</sup> Although this variability results to some extent from the choice of patient group, much is likely to be due to the choice of assay method. The frequency and levels of these antibodies fluctuate with disease activity.<sup>8,9</sup>

ELISA has been used as a sensitive method for detection of anti-dsDNA antibodies.<sup>10</sup> This technique detects both low and high avidity antibodies although the structure of DNA may be altered when DNA interacts with a solid support. This makes solid phase based assays questionable for clinical use, at least as the sole anti-DNA assay.<sup>11</sup> *Crithidia luciliae*, on the other hand, is a frequently used technique for detecting anti-nDNA, combining high sensitivity with high disease specificity.<sup>12</sup> This microorganism has a giant mitochondrion which contains pure native DNA and it is the fluorescence of this which constitutes a positive test. Comparison studies of anti-DNA antibody measuring assays have been performed by several investigators.<sup>10,13</sup>

To evaluate the diagnostic meaning of ELISA and IF techniques for measuring dsDNA. We simultaneously tested 110 ANA positive sera from SLE patients, RA and other non-specific disease control patients for the presence of antibodies to dsDNA using both assays.

## PATIENTS AND METHODS

*Patient Sera:* One hundred and ten consecutively collected sera which had been tested positive for ANA were included in this study. These sera were derived from 40 patients with SLE, 30 patients with RA and from non-CTDs as disease controls (n=40). Thirty sera from normal healthy subjects were also included as negative controls.

*Anti-Nuclear Antibody (ANA) Test:* ANA was determined by immunofluorescence using a commercial kit (Hep-2 Slides; The Binding Site Limited, Birmingham-UK). Hep-2 cells were fixed on slides and were used as the substrate. Patient's sera were incubated with the substrate, the slides were then washed three times and antigen-antibody reaction was detected by FITC-labeled anti-human gamma globulin conjugate. All slides were read using a fluorescence microscope. In this study, sera were considered positive when fluorescence was detected at dilutions of 1:40 or higher.

*Anti-dsDNA Antibody Assays:* All the assays used in this study were configured to detect IgG antibodies only.

a. *Anti-dsDNA Antibody by Enzyme Immunoassay (ELISA) Method:* Anti-dsDNA antibody by ELISA was determined by using Bindazyme Human Anti-dsDNA EIA Kit (a commercial kit from the Binding Site Limited, Birmingham-UK). In brief, a 96-well microtiter plate previously coated with highly purified dsDNA was used. In each well, a 100 microliter amount of 1:101 diluted patient sera was added; after incubation, each plate was washed three times. To each well, a 100 microliter amount of alkaline phosphatase conjugated anti-human IgG conjugate was added; after incubation and washing, a substrate was added and the colour development was read at 405 nm. The results are expressed in IU/mL by using calibrators provided in the kit. A value greater than 75 IU/mL was considered positive.

b. *Anti-dsDNA Antibody by Indirect Immunofluorescence (Crithidia lucilliae) Method:* The detection of anti-dsDNA antibodies in sera by indirect immunofluorescence test was performed using the INOVA Diagnostics Anti-dsDNA Antibody Test System (San Diego, CA-USA). The test employs the hemoflagellate, *Crithidia lucilliae*, as a substrate. This single celled organism possesses a giant mitochondrion containing a highly condensed mass of circular dsDNA. This mass of circular dsDNA, known as the kinetoplast, appears to be free of histones or other nuclear proteins. It serves as a sensitive and specific substrate for detecting antibodies for dsDNA. Standard indirect immunofluorescent techniques were used. In brief, *Crithidia lucilliae*, fixed on wells on a glass slide, was used as the substrate and 1:10 diluted patient serum was added to each well. After incubation, it was washed three times and antigen-antibody reaction was determined by FITC-labeled anti-human IgG. A positive test was considered at a titer of 1:10 or above.

*Statistics:* All analyses were performed with the use of SPSS (version 11.0). Resulting

p values <0.05 were considered to indicate significance.

### RESULTS

*Anti nuclear Antibody (ANA) Test:* All sera from SLE, RA and disease control groups included in this study tested positive for ANA IF assay. Only 2/30 sera derived from normal healthy subjects gave positive ANA results.

*Enzyme-Linked Immunosorbent Assay:* The results of the ELISA assay on all 140 sera are shown in Table-I and Figure-1. A cut-off value of greater than 70 IU/mL was observed, sera from SLE patients showed 24/40 (60%), from the RA group 5/30 (16.6%), from the disease control group 9/40 (22.5%) and from normal healthy subjects 1/30 (3.3%) positive for anti-dsDNA antibodies.

*Crithidia lucilliae Indirect Immunofluorescence (IF) Assay:* The results of the IF test on 150 sera are shown in Table-I and Figure-1. SLE group showed positive results in 16/40 (40%), RA in 3/30 (10%) and disease control sera in 5/40 (12.5%). None of the normal healthy controls were positive for anti-dsDNA IF assay.

*Summary of Anti-dsDNA Detected by the Two Methods:* In the present study a clear distinction between SLE and other groups was found with the anti-dsDNA antibodies assayed by ELISA and IF methods (P<0.05). Similarly, all groups (SLE, RA and disease control) showed a significantly higher levels (P<0.001) of anti-dsDNA antibodies as measured by either ELISA or IF methods, when compared to that of normal healthy group.

Table-I: Comparison of the number of sera with raised anti-dsDNA in SLE, patients with RA, ANA+ disease control and normal healthy controls.

	ELISA	IF
SLE (n=40)	24/40 (60%)	16/40 (40%)
RA (n=30)	5/30 (16.6%)	3/30 (10%)
Disease control (n=40)	9/40 (22.5%)	5/40 (12.5%)
Healthy control (n=30)	1/30 (3.3%)	0/30 (0%)

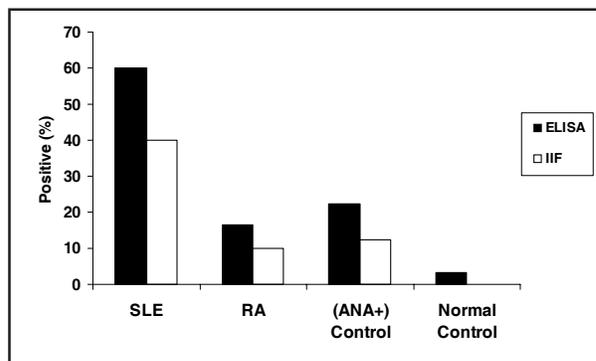


Fig-1: Comparison of the number of sera with raised anti-dsDNA in SLE, RA , ANA + disease control and normal healthy controls.

A negative anti-dsDNA antibody result determined by ELISA and positive result by IF assay was seen in 2/40 (5%), 2/30 (6.6%) and 1/40 (2.5%) sera from SLE, RA and disease control groups respectively. Positive ELISA tests and negative IF were seen in 9/40 (22.5%), 4/30 (13.3%) and 5/40 (12.5%) sera from SLE, RA and the disease control group respectively; this shows slightly less sensitivity of the IF assay. A positive result by ELISA and IF assay was seen in 14/40 (35%), 1/30 (3.3%) and 4/40 (10%) sera from SLE, RA and disease control groups respectively, suggests low avidity anti-dsDNA was detected by the ELISA and IF assay.

### DISCUSSION

The importance of antibodies to dsDNA in the diagnosis and management of SLE has been universally recognized and used in daily clinical practice. However, the reliability of anti-dsDNA antibody detection by any given method is dependent upon numerous variables such as purity of DNA substrate, avidity and/or affinity of antibody, distinction between binding of antibodies versus non-immunoglobulin proteins, isotypes of antibodies and finally, complement-fixing properties in a complement-dependent method.<sup>14</sup> Antibodies to dsDNA are very heterogeneous with regard to their avidity, their isotype (class), and their ability to cross-react. Considering all the variables and multiple epitopes of the DNA

molecule, none of the methods are absolutely specific and sensitive.

Our results indicate that sera which is anti-dsDNA positive by one method does not necessarily yield a positive result by other methods. Antibodies to single stranded DNA or other constituents used in manufacturing processes are known to give false positive results in dsDNA ELISAs.<sup>15</sup> Lipoprotein-IgG complexes are a major cause of false positive results in IF.<sup>15</sup> In addition, antibodies to histone and other DNA related/associated proteins (deoxyribonucleic proteins, DNPs) can result in false positives in all the test systems mentioned above.<sup>15,16</sup> The specificity of the IF assay is high but low sensitivity; this was confirmed in this study.

All of the tests including ELISA and IF are a compromise between specificity and sensitivity. Exact numbers vary from test system to test system and from manufacturer to manufacturer, but in general, ELISA is the most sensitive followed IF assay.

When comparing ELISA to IF for the detection of dsDNA, the current study results were in agreement with several earlier reports<sup>10,17,18</sup> that the ELISA technique is more sensitive resulting in a higher positivity percentage. Our results are also similar to those obtained by others<sup>17,18</sup> who measured dsDNA with IF technique on groups of patients similar to those employed in our study.

Several studies have compared the measurement of anti-dsDNA antibodies by the ELISA and the *Crithidia luciliae* methods. Results indicated that the ELISA is more sensitive than the *Crithidia luciliae* IF assay, yet in this study, *Crithidia lucilliae* (IF) assay showed a comparable detection rate of anti-dsDNA to that measured by the ELISA in all groups tested, this is in agreement with other reported studies.<sup>7,11</sup> Earlier investigations, after observing such a strong correlation between the two techniques, proposed that ELISA may be a useful alternative to the *Crithidia* assay.<sup>17,18</sup>

In most studies, specificity of IF has been reported to be greater than other methods.<sup>19</sup>

One reason for this is that the kinetoplast of *Crithidia luciliae*, is full of dsDNA and lacks single-stranded DNA.<sup>20</sup> There are different reports about the sensitivity of IF.<sup>11,19</sup> In this regard, the method of detection and the dilution factor is important. In our study sera were diluted 1:10, and this may be the cause of low sensitivity of IF observed over the ELISA.

The other advantage of the IF assay is the highly stable, double stranded, concentrated DNA in a kinetoplast, thus giving very high specificity for anti-dsDNA detection. The main disadvantages are that it is subjective, semi quantitative and has relatively low sensitivity. A false positive due to anti-histone antibodies<sup>21</sup> and kit-to-kit variability due to preparation of *Crithidia lucilliae* substrate has also been reported.<sup>22</sup>

## CONCLUSIONS

In conclusion our findings confirm other reports that ELISA is more sensitive than IF on *Crithidia luciliae* for detection of anti-dsDNA antibodies. However, there is a good correlation between the results obtained by ELISA and IF techniques in detecting anti-dsDNA antibodies. Since the ELISA is easy to perform, and can easily be automated, these characteristics should allow its rapid application to the clinical routine, as a useful alternative to the *Crithidia* assay or, at least, an effective screen prior to testing in the more technically difficult and time consuming assays for the measurement of anti-dsDNA antibodies.

Our data also showed unique combinations of anti-dsDNA antibodies in association with the Omani patient groups tested. Employing both ELISA and IF methods, we demonstrated a substantial overlap of these antibodies in the patients selected which may reflect different pathogenic interactions between anti-dsDNA antibodies, their target antigens, and the target organ environment at the molecular level. The presence of anti-dsDNA antibodies in RA and other positive control disease groups suggests that these antibodies may not be unique to SLE.

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