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DETERMINATION OF ANTI-dS-DNA ANTIBODY LEVELS IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS BY ELISA, RIA AND CRITHIDIA METHODS: CHANGES IN ANTIBODIES PRIOR TO DISEASE ACTIVITY

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- **ABSTRACT**

Background: Antibodies (Abs) to double-stranded (ds) DNA have been considered most useful in diagnosis and monitoring disease activity in patients with systemic lupus erythematosus (SLE).
Objective: To investigate the value of anti-ds-DNA Ab level changes, detected by three different methods, as predictor of disease activity in SLE patients.
Methods: Serial sampling from 87 patients with SLE was performed. Samples were assessed for anti-ds-DNA Abs by Crithidia luciliae test (CLT), ELISA and Farr assay.
Results: In 17 out of 40 cases with completed sampling, disease activity was occurred during the study. Significant increases in anti-ds-DNA Ab level was detected by ELISA (whole and IgG) and Farr assay prior to disease activity in all clinically active patients. CLT was tested positive in only one case before disease activity. None of the 23 cases with clinically inactive disease were tested positive with CLT,whereas in 3 and 15 of 23 cases positive results were observed by ELISA and Farr assay,respectively . Statistical analysis showed the predictive value of ELISA for disease activity. There was a high correlation between the results of Farr assay and ELISA for all SLE patients in detecting anti-ds-DNA Abs ($r=0.63, p< 0.0001$). ELISA and Farr assay were also correlated well with disease activity ($p<0.05$).
Conclusion: Our findings confirm the predictive value of increases in anti-ds-DNA Abs measured by ELISA method with respect to activity of SLE.

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Key Words • Systemic lupus erythematosus • antibodies, anti DNA • ELISA

Introduction

Antibodies to ds-DNA are found in the sera of SLE patients, and are considered as a marker of disease activity.[1-3] These Abs not only are considered to be of diagnostic significance but also are considered to be of prognostic value.[4] The prognostic value is illustrated by the finding that, in individual patients, fluctuations in Ab level tend to correlate with course of disease.[5,6] Several reports have studied the changes in anti-ds-DNA Ab level instead of absolute values and find that increases in anti-ds-DNA Ab level may act as a predictor of disease activity and exacerbation.[7-9] This prospective study was undertaken to find a probable correlation between disease activity and changes in the levels of anti-ds-DNA Abs. Since these Abs are a heterogeneous group with different characteristics, their measurement was assessed using three different methods including immunofluorescence with Crithidia luciliae (CL), enzyme linked immunosorbent assay (ELISA) and radioimmunoassay (RIA). Correlation of each method with disease activity, C3 and C4 changes and clinical manifestation were also investigated.

Materials and Methods

Two hundred and one sera from 87 patients with SLE and 110 sera from controls including patients with rheumatoid arthritis and other non-SLE autoimmune diseases (60 cases) and normal healthy donors (50 cases) were obtained in the Department of Internal Medicine, Shiraz University of Medical Sciences.

All SLE patients fulfilled the diagnostic criteria of the American College of Rheumalology.[10] Their

mean age was 29 years (range 15-48) and the male female ratio was 7:80. In 40 SLE patients 3 to 5 samples were obtained once every three months.

The clinical activity of all patients was assessed according to the protocol reported previously by Bombarier.[10]

Patients were divided into three groups of inactive, moderately active and severely active. Major and minor excerbations were defined for patients. Sera of SLE and non-SLE patients and normal controls were assessed for anti-ds-DNA Abs by CLT and ELISA. Anti-ds-DNA Abs were also detected by RIA in SLE patients.

CLT assay:

Crithidia organisms (University of Tarbiat Modarres, Department of Parasitology, Tehran) were grown in RPMI 1640 medium containing 5% fetal calf serum (Gibco, Germany). A standard indirect immunofluorescence technique was employed using slides prepared with Crithidia. FITC-conjugated rabbit-antihuman whole Ig and an FITC-labeled rabbit antihuman IgG (Dako, Denmark) were used for staining. Serum specimens were tested in serial dilutions. A dilution of 1:10 or greater was regarded as positive.

ELISA method:

In order to detect anti-ds-DNA Abs, an ELISA method was developed by preparing ds-DNA from calf thymus as previously described.[11] Briefly, calf thymus was frozen, minced and then homogenized. After lysis of red blood cells and precipitation of proteins and lipids, propanol was added to extract the DNA. DNA was dissolved in ionized water. After1 hr RNase -A (0.1 ?g/ml) was added to remove RNA. The ratio of optical densities (OD) of the obtained solution at 260 to 280 nm was greater than 1.8. Prepared DNA was compared with standard calf thymus DNA (Worthington, USA) by electrophoresis. No additional bands were observed. Ninty-six well microtiter plates (Nunc, Denmark) were pre-coated with 100 ?l of protamine sulfate (0.5 ?g/ml) per well. DNA diluted to 2 ?g/ml was added to the plates. After 24 hrs incubation at 4 ? C, sera were diluted 1:50 and added to each well in triplicate. Then HRP-conjugated goat anti- human IgG or HRP-conjugated anti-human whole Ig (Dako, Denmark) was added. After addition of the substrate, absorbance was read at 492 nm by an ELISA reader (Pharmacia, Sweden). OD of normal control sera was analyzed to obtain the cut off point (mean + 2SD). Samples with OD greater than the cut off point were regarded as positive.

RIA (Farr assay):

The method was performed using ^{125}I-ds-DNA (Amersham Co., Germany). 25 ?l of Sera was incubated with 200 ?l of ^{125}I-dsDNA. After incubation for 1 hr at 37 ? C, the reaction was stopped by the addition of saturated ammonium sulfate. The mixture was centrifuged and the precipitate was counted in a liquid scintillation counter. Samples with more than 7 IU/ml were considered positive.

Complement assay:

C3 and C4 levels were measured by single radial immunodiffusion (Baharafshan, Tehran, Iran).

Statistical analysis was performed by SPSS software using student's t, Fisher's exact and Chi-square tests. Spearman correlation coefficient was calculated for possible correlation between study parameters.

Results

Detection of anti-dsDNA Abs:

Results of ELISA and CLT for whole and IgG anti-ds-DNA Ab levels in disease control groups and in SLE patients regardless of clinical status are shown in Table 1. By ELISA only 4 of 60 non-SLE disease control sera and 2 of normal healthy individual sera were positive. The specificity of the ELISA method was 93% for detection of whole anti-dsDNA and 95% for IgG anti-dsDNA. The ELISA was found to be more sensitive than CLT and less sensitive than Farr assay, as 62% of SLE sera were positive for whole anti- ds-DNA Abs and 55% for IgG Abs. Strong statistically significant differences between detection of Abs in SLE versus rheumatoid arthritis and other non-SLE sera were obtained by this method ($p<0.01$). Although no false positivities was found in any of the control groups by CLT, it showed a low sensitivity as only 12% of SLE patients were tested positive by this method, and no significant difference between the SLE and disease controls was found. Study of sera by RIA method showed 79% positivity among SLE patients. A high correlation was found between the results of RIA and ELISA for detection of anti-ds-DNA Abs ($r=0.63$, $p<0.0001$)

Levels of anti-dsDNA Abs in relation to disease activity:

As shown in Table 2, of 103 sera with active disease, 91 sera were positive with RIA, 85 by ELISA (whole Ab) and 24 by CLT (whole Ab) assay. The majority of positive cases with CLT were in clinically severe active disease (91%). This was observed by RIA and by ELISA in 35% of cases. Less than 7% of disease control group, 82.5% of SLE sera with active disease, and 49% of SLE sera with inactive disease showed positivity by ELISA technique (Fig 1). Statistical analysis showed significant correlation between disease activity and IgG ELISA ($p=0.05$), whole ELISA ($p=0.01$) and RIA ($p=0.05$).

C3 and C4 levels during disease activity:

Decreases in C3 and C4 levels occurred in 90 and 40 of 201 sera respectively. Contrary to C3 level, statistical analysis showed a significant correlation between decrease in C4 level and disease activity ($p<0.001$). An association between decrease in C4 level and positivity for anti-ds-DNA Ab was found by all three methods ($p<0.05$).

Anti-ds-DNA antibody and clinical manifestations:

Arthritis was the most common manifestation among SLE patients. All patients with kidney or CNS involvement had at least one positive result of anti-ds-DNA Ab by one of the three methods. Analysis of lupus patients by sub-dividing them into those with and without the major clinical features showed no significant correlation with various assay methods.

Specificity of changes in anti-ds-DNA antibody levels for predicting disease activity:

In order to find the predictive value of anti-ds-DNA levels for disease activity serial prospective studies of SLE patients sera were performed. All clinically active patients had a preceding significant increase in anti-ds-DNA Ab levels detected by ELISA and RIA methods. Only in one case before an exacerbation a positive result by CLT was observed. None of the 23 cases with clinically inactive disease showed positive results with CLT, whereas positivity in 3 and 15 of 23 cases was observed by ELISA and RIA, respectively. Statistical analysis showed a predictive value of ELISA method for disease activity. Fig. 2 shows

changes of anti-ds-DNA Abs determined by ELISA method with disease activity in one patient.

Discussion

In the present study a clear separation between SLE and non-SLE control groups was found with the anti- ds-DNA IgG and whole ELISA methods (P<0.01). ELISA assay has been introduced as a sensitive method for detection of anti-ds-DNA Abs.[12, 14] This is based on the

finding that by this technique both low and high avidity antibodies are detected.[15] In most studies, specificity of CLT has been reported to be greater than other methods.[16] One reason for this is the kinetoplast of CL, which is full of ds-DNA and lacks single-stranded DNA.[17] There are different reports about the sensitivity of CLT.[15,16] 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 CLT.

Measurement of anti-dsDNA is widely used to assess disease activity in SLE.[18,19] In our study levels of these Abs in relation to disease activity, indicated that RIA is the most sensitive method and CLT the most specific one, as none of the patients in clinically inactive form showed a positive result with the CLT method. In this respect, a significant correlation between RIA and ELISA methods (r=0.63, p<0.001) suggests that ELISA is a sensitive method which may be used instead of RIA for assessing disease activity.

No significant differences between IgG and whole anti-ds-DNA Abs in ELISA and CLT assays was observed.

Measurement of complement components in SLE patients has been widely investigated.[19,20] Decreased C3 levels as well as C4 levels, have been reported to be associated with active disease especially with renal involvement.[20] In the present study, significant correlation between decreases in C4 levels, disease activity and anti-ds-DNA Abs was observed (p<0.05). This result was expected since most of our patients were in early lupus activity.

In some previous studies, the Farr assay has been reported to detect predominantly high avidity anti-ds-DNA Abs, the CLT assay Abs of intermediate avidity, and the ELISA both high and low avidity Abs.[21] High avidity anti-ds-DNA Ab has been presumed by some investigators to be associated with renal disease and low avidity anti- ds-DNA Abs with cerebral involvement.[15] This data is not confirmed by others.[22] In the present study, we did not find any association between clinical symptoms and anti-ds-DNA Abs.

Our study, comparing three assays with respect to their ability to predict disease activity, indicated that ELISA is the best method. Some authors have reported that both RIA and ELISA methods are valuable techniques.[5,7] Borg claimed that the Farr assay had the highest sensitivity and may be considered as the method of choice for predicting exacerbation of SLE, and that ELISA is an alternative to the Farr assay.[7]

In conclusion our findings confirm other reports on the predictive value of increases in anti-ds-DNA Abs with respect to activity of SLE. Measurement of these Abs by ELISA, a sensitive method which does not require the equipment used to perform the Farr assay, is valuable for assessing or predicting disease activity in SLE.

It is recommended that testing of anti-ds-DNA Abs at least every three months and pre-treatment of patients who show an increase in anti-ds-DNA levels is useful in the prevention of active disease and tissue damage in patients with clinically silent disease.

References

1. Rahman MA, Isenberg DA. Autoantibodies in systemic lupus erythematosus. *Curr Opin Rheumatol 1994;* **6:**468-73
2. Isenberg DA, Rahman MA, Ravirajan CT, Kalsi JK. Anti-DNA antibodies: from gene usage to crystal structures. *Immunol Today 1997;***18:**149-53.
3. Chretien P, Dauvin M, Hellen P, et al. Comparison of indirect immunofluorescence on Crithidia luciliae, Farr test, and immunoenzymatic methods for the screening of anti-native DNA autoantibodies. *Ann Biol Clin Paris 1994;* **52:**645-50.
4. Pisetsky DS. Anti-DNA antibodies in systemic lupus erythematosus. *Rheum Dis Clin North AM 1992;***18:**437-54
5. Ting CK, Hsieh KH. A long-term immunological study of childhood onset systemic lupus erythematosus. *Ann Rheum Dis 1992;***51:**45-51.
6. Smeenk RJ, Van den Brink HG, et al. Anti-dsDNA: choice of assay in relation to clinical value. *Rheumatol Int 1991;***11:**101-7.
7. Ter Borg EJ, Horst G, Hummel EJ, et al: Measurement of increases in anti-double-stranded DNA antibody levels as a predictor of disease exacerbation in systemic lupus erythematosus. *Arthritis and Rheum 1990;***33:**634-43
8. Ter Borg EJ, Horst G, Hummel EJ, et al: Rises in anti-double stranded DNA antibody levels prior to exacerbations of systemic lupus erythematosus are not merely due to polyclonal B cell activation. *Clin Immunol Immunopathol 1991;***59:**117-28.
9. Bootsma H, Spork PE, Ter Borg EJ, et al: The predictive value of fluctuations in IgM and IgG class anti-dsDNA antibodies for relapses in systemic lupus erythematosus. A prospective long term observation. *Ann Rheum Dis 1997;***56:**661-6.
10. Bombarier C, Gladman DD, Urowitz MB: Derivation of the SLEDAI, a disease activity index for lupus patients. *Arthritis Rheum 1992;***35:**630-40.
11. Graham DE. The isolation of high molecular weight DNA from whole organisms or large tissue masses. *Analytical Biochem 1978;***85:**609-13.
12. Tzioufas AG, Terzoglou C, Stavropolos ED, et al: Determination of anti-dsDNA antibodies by three different methods: Comparison of sensitivity, specificity and correlation with lupus activity index (LAI). *Clin Rheumatol 1990;***9:**186-92.
13. Eaton RB, Schnneider G, Schur PH: Enzyme immunoassay for antibodies to native DNA. *Arthritis and Rheum 1983;***26:**52.
14. Tipping PG, Buchanan RC, Riglar AG, et al: Measurement of anti-DNA antibodies by ELISA: A comparative study with Crithidia and a Farr assay. *Pathology 1991;***23:**21-4.
15. Isenberg DA, Dudeney C, Williams W, et al: Measurement of anti-DNA antibodies: a reappraisal using five different methods. *Annal Rheum Dis 1978;***46:**448-56.
16. Isenberg DA, Ehrenstein MR, Longhurst C, et al: The origin, sequence, structure and consequences of developing anti-DNA antibodies. A human prospective*,Arthritis Rheum 1994;***37:**169-80.
17. Shapiro TA, Englund PT: The structure and replication of kinetoplast DNA. *Annu Rev Microbiol 1995;***49:**117-43.
18. Brnkman K, Termaat R, Berden JHM, Smeenk RJT: Anti-DNA antibodies and lupus nephritis. The complexity of cross reactivity. *Immunol Today 1990;***11:**232-4.
19. Suzuki N, Mihara S, Sakane T: Development of pathogenic anti-DNA antibodies in patients with systemic lupus erythematosus. *FASEB 1997;***11:**1033-8.
20. Hilliquin P: Biological markers in inflammatory rheumatic diseases. *Cell Mol Biol 1995;***41:**993-1006.
21. Taman, Yabuki T, Furuyoushi S, et al: Studies on anti-dsDNA antibodies associated with renal involvement by enzyme-linked immunosorbent assay in patients with systemic lupus erythematosus. *Ryumachi 1996;***36:**8-15.
22. McGrath H, Biundo JJ Jr: A longitudinal study of high and low avidity antibodies to double-stranded DNA in systemic lupus erythematosus. *Arthritis Rheum 1985;***28:**425-30.

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