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Opinion Article

# IgG, IgM, and IgA Antinuclear Antibodies in Discoid and Systemic Lupus Erythematosus Patients

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## ABOUT THE STUDY

Patients with Systemic Lupus Erythematosus (SLE) have higher levels of IgG Antinuclear Antibodies (ANAs) than those with Discoid Lupus Erythematosus (DLE). We evaluated the expressions of IgG, IgM, and IgA ANAs in DLE and SLE patients to provide an expanded immunologic picture of circulating ANAs in lupus patients. Using Enzyme-Linked Immunosorbent Assays (ELISAs) and Indirect Immunofluorescence (IIF) using monkey oesophagus as substrate, sera from age, gender, and ethnically matched SLE, DLE, and normal patients were evaluated for IgG, IgM, and IgA ANAs. When compared to patients with DLE and healthy individuals, ELISA results for SLE patients indicated greater levels of IgG ANA, IgM ANA, and IgG/IgM ANA ratios. In comparison to normal patients, the expression of IgA ANA was greater in SLE and DLE patients.

In IIF trials, patients with SLE had greater rates of IgG, IgM, and IgA ANA positivity. IgG/IgM ANA ratios that are higher in SLE than DLE indicate greater class-switching and a longer-lasting humoral response. They also hint at a possible link between IgM ANAs and disease control. Systemic Lupus Erythematosus (SLE) and Discoid Lupus Erythematosus (DLE) may or may not coexist, with DLE developing into SLE in 17% of individuals with SLE and occurring in 20% of SLE patients. Through measurements of the amounts of circulating IgG autoantibodies, more distinctions between the two disorders have been made. According to earlier research, SLE patients have higher levels of IgG Antinuclear Antibodies (ANAs) than DLE patients. It is unknown, though, whether IgM or IgA ANA levels can also discriminate between people with DLE and SLE. We used Enzyme-Linked Immunosorbent Assays (ELISAs) and indirect immunofluorescences to examine the expressions of IgG, IgM, and IgA ANAs in patients with DLE and SLE in order to better understand the immunologic linkages between the two diseases. We predicted that the ANA concentrations for all three people with SLE would have the greatest isotypes, followed by those with DLE and normal patients.

The Dallas Regional Autoimmune Disease Registry or the UTSW Cutaneous Lupus Registry. The UTSW Institutional Review Board gave its approval for the study, which was carried out in accordance with the Declaration of Helsinki's code of ethics. Three groups of patients were created: SLE, DLE, and normal, which were matched for age, gender, and ethnicity. While DLE patients had a DLE diagnosis based on clinicopathologic correlation and less than four ACR SLE criteria, SLE patients met at least four of the American College of Rheumatology's (ACR) SLE diagnostic criteria. If the healthy controls had a history of autoimmune disorders, they were omitted. Each patient's demographic information, medical background, and clinical data were gathered. Additionally, Cutaneous Lupus Erythematosus was used to gauge the degree of systemic and cutaneous disease activity in each DLE and SLE patient. ELISAs were carried out using commercially available ELISA kits to measure IgG, IgM, and IgA ANAs (INOVA Diagnostics, Inc., San Diego, CA). The ELISA techniques for IgM and IgA ANA included horseradish peroxidase-conjugated goat anti-human IgM (1: 4,000 dilution) or IgA (1: 5,000 dilution) second-step antibodies. ELISAs for IgG were carried out in accordance with the manufacturer's instructions (Jackson Immuno Research Laboratories Inc., West Grove, PA). IgM and IgA OD450 values were measured, and by extrapolating the OD450 values to a standard curve, concentrations of IgG ANAs were estimated.

In a humidified environment, patient sera (1:20 dilution) were incubated with six-micron-thick cryosections of monkey esophageal tissue for 30 minutes. This was followed by three five-minute washes with 1X PBS. Two blinded researchers analysed the results of tissue cryosections that had been treated with fluorescein isothiocyanate-conjugated goat anti-human IgG (1:80 dilution), IgM (1:40 dilution), or IgA (1:80 dilution) (Invitrogen, Carlsbad, CA) and washed in the same way. We compared patient characteristics using Fisher's exact test or chi-squared test for categorical variables and Student's test or one-way Analysis of Variance (ANOVA) for continuous data. We utilised the Kruskal-Wallis test and Dunn's multiple comparisons post hoc test to analyse ELISA results. The Fisher's exact test or the chi-squared tests were used to compare the percentages of positive IIF results. The IgG, IgM, and IgA ANAs' differential expression in DLE and SLE patients, which gives a comprehensive picture of the many ANA isotypes present in these lupus subtypes. Reduced IgG/IgM ratios between DLE and SLE point to enhanced IgM to IgG class switching in SLE and a link between IgM ANAs and disease prevention. Increased IgA ANA levels in DLE patients compared to normal controls may indicate IgA is involved in the pathogenesis of DLE in some way.

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