Original article

Antinuclear antibodies in suspected Systemic lupus erythematosus (SLE) patients of a tertiary care hospital- a retrospective study

Asha Patil1, Chaitra2, Deepa Kamath3, Indira Bairy4, Kusumakshi Nayak5

Abstract

Background: In a systemic autoimmune disorder, non-organ-specific autoantibodies binding to the antigens are expressed within the human cells. The presence of antinuclear antibody (ANA) in the blood indicate an autoimmune disease. Objective: The study aims to estimate the frequency and prevalence of ANA in suspected autoimmune disorders in a tertiary care hospital.

Materials and methods: A retrospective study and the ANA test results data obtained from the Laboratory Information System and laboratory records. Result: The frequency of the ANA test in six months was 5879; among them, 1638 (27.8 %) had ANA positive test either global or profile with female predominance (2:1). ANA positive test selected for further analysis was 401, in which 154(38%) were from the age group of 20 to 40 years. Conclusion: The estimated prevalence of ANA positive tests was 27.8%, which differed in different sex and age groups. In autoimmune conditions, estimating the prevalence of ANAs influences the trends of antinuclear antibodies. The test frequency and its interpretation assist in patient diagnosis and management, along with specific diagnostic tests.

Keywords: ANA; dsDNA; SLE; lupus; autoimmune disorder

Short title: ANA in SLE patients

Introduction

In systemic autoimmune disorder, non-organ-specific autoantibodies bind to one or more antigens that are expressed within the nucleus of human cells. An antinuclear antibody (ANA) in the blood can indicate the possibility of an autoimmune disease.1 Normally, sequestered DNA antigen is not accessible to the immune system. However, in certain circumstances, such as infection, tissue injury, and apoptotic DNA bound microparticles are recognized by anti-DNA antibodies or ANA. The subsequent consequences amplify autoreactive B cells, resulting in high-affinity antibodies and increased activated T cells2. These autoantibodies are associated with various autoimmune diseases such as systemic lupus erythematosus (SLE), scleroderma (SSc), polymyositis/dermatomyositis (PM/DM), and rheumatoid arthritis (RA) and Sjögren’s syndrome.

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(SjS)³. Anti-DNA antibodies can be present in serum as a predictor of lupus before diagnosing clinical disease².

ANA is one of the biomarkers for systemic lupus erythematosus evaluation and is the most preferred test for autoimmune disorder assessment by clinicians. However, positive ANA results can be seen in an average of 30% of healthy people⁴. ANA is important in screening, diagnosis, treatment evaluation, or monitoring the prognosis of autoimmune disorders. ANA screening is requested in suspected autoimmune diseases with clinical symptoms or laboratory results³. The presence of ANA, along with appropriate clinical history, helps in accurate diagnosis of disease⁶. ANA positive SLE patients have chronic diseases of multisystem involvement. Conversely, the presence of ANA in the blood is nonspecific because these antibodies may present in healthy individuals and infectious disease cases in lower titers, not necessarily indicating autoimmune diseases. The frequency of ANA-positive reports is found to be >20%⁷. Since ANA-positive consequences in dysregulation of the immune system differ from SLE, these healthy individuals eventually may not develop autoimmune disease, or few may produce disease⁸.

Indirect immunofluorescence (IIF) is the best method with high sensitivity and specificity in screening suspected SLE cases⁹,¹⁰. Antinuclear antibodies targeting the nucleus of a cell typically include antibodies to nuclear materials, DNA, and histones⁹. In ANA global method, autoantibodies interact with cellular components (protein, dsDNA & RNA). They are detected on mammalian cells on slide¹. The human sera will be incubated with fixed cells, and fluorescein isothiocyanate (FITC) conjugated antibodies to human IgG. Titre of autoantibodies are observed under a fluorescent microscope¹¹. In ANA profile, an immunoblotting method with autoantibodies on EUROLINE test strips. The test strip has coated antigens such as nRNP, Sm, SSA, SS-B, Scl-70, PM-Scl, Jo-1, CENP B, PCNA, ds DNA, nucleosomes, histones, ribosomal P- protein, AMA M2. Patient samples are incubated with immunoblot strips in the first step of the reaction. Specific IgG antibodies (also IgM and IgA) will bind to the corresponding antigenic site in the positive samples. A second incubation is performed to detect bound antibodies using an enzyme-labeled anti-human IgG (enzyme conjugate) that catalyzes a color reaction. This retrospective study aimed to assess the frequency and prevalence of ANA in suspected SLE patients in a tertiary care hospital¹²,¹³.

**Materials and methods**

A retrospective study was conducted in a tertiary care hospital, Manipal, to evaluate the presence of ANA’s in suspected SLE cases with clinical symptoms such as arthritis, hemolytic anemia, renal disease, pneumonitis, and other pulmonary manifestation, thyroid dysfunction, liver, and gastrointestinal symptoms, skin rashes, along with other nonspecific symptoms like fever, malaise, fatigue, arthralgia, and myalgia. ANA data were collected from laboratory records from August 2016 to July 2017. ANA global and ANA profile serology tests performed by IIF and Immunoblotting methods (kit method) in suspected SLE patients. The results were expressed in frequency and percentage.

**Ethical clearance: The study was accepted by the Institutional Research Committee (IEC 209/2017).**

**Results**

The frequency of ANA testing during a six-month study was 5879. Among them, 1638 (27.8%) were positive either for the ANA global or ANA profile. Four hundred one cases irrespective of ANA global or profile positive selected for further analysis, revealed that 268 (67%) were female, and 133 (33%) were male, the female to male (2:1). The majority of positive cases were between 21 to 40 age groups (154 patients -38%) and 90 cases (22%) between the 41 and 50 age groups. (Table 1).

**Table 1: Frequency of favorable results in different age groups and gender.**

<table>
<thead>
<tr>
<th>Age range (Range (years))</th>
<th>Number (n=401)</th>
<th>Percentage (%)</th>
<th>Male (n=133)</th>
<th>Female (n=268)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-10</td>
<td>8</td>
<td>2</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>11-20</td>
<td>29</td>
<td>7.2</td>
<td>8</td>
<td>21</td>
</tr>
<tr>
<td>21-30</td>
<td>78</td>
<td>19.5</td>
<td>21</td>
<td>57</td>
</tr>
<tr>
<td>31-40</td>
<td>76</td>
<td>19</td>
<td>21</td>
<td>55</td>
</tr>
<tr>
<td>41-50</td>
<td>90</td>
<td>22.5</td>
<td>25</td>
<td>65</td>
</tr>
<tr>
<td>51-60</td>
<td>64</td>
<td>16</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>61-70</td>
<td>38</td>
<td>9.4</td>
<td>16</td>
<td>22</td>
</tr>
<tr>
<td>71-80</td>
<td>11</td>
<td>2.7</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>&gt;81</td>
<td>7</td>
<td>1.7</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

Out of 401 samples, 242 were ANA global test positive, and the intensity of the ANA antibodies was graded 1+ (n=125), moderately positive 2+ (n=69),
strongly positive 3+ (n=48). Among these, 232 cases had antibodies to the nucleus and 98 cases to the cytoplasm. Fifty-four cases showed antibodies to both the nucleus and cytoplasm. ANA profile subset test was performed in suspected autoimmune disease. (Table 2) Predominantly it showed that the positive-frequency autoantibodies were to nRNP (5.9%), SS-A (RO) Sjogren’s syndrome type A (6.7%), and Sm (Smith) (4.7%). The autoantibodies with the positive frequency were anti-Ro-52 (19%), anti-M2 (17.8%), and anti-SSA (14.3%), whereas anti-Scl-70, anti-Jo-1, and anti-Sm were the less frequently detected antibodies among the autoantibodies. Only 23 cases showed titer-positive dsDNA between 190 and > 800U/ml.

Table 2: Antinuclear antibodies and their prevalence in our setup.

<table>
<thead>
<tr>
<th>ANA SUBSET</th>
<th>Percentage (n=401)</th>
</tr>
</thead>
<tbody>
<tr>
<td>nRNP (anti-nuclear ribonucleoprotein)</td>
<td>5.9%</td>
</tr>
<tr>
<td>Sm(Smith)</td>
<td>4.7%</td>
</tr>
<tr>
<td>SS-A (RO) Sjogren’s syndrome type A</td>
<td>6.7%</td>
</tr>
<tr>
<td>SS-B(Sjogren’s syndrome type B)</td>
<td>2.4%</td>
</tr>
<tr>
<td>Scl-70(scleroderma)</td>
<td>1.9%</td>
</tr>
<tr>
<td>Pm-scl (polymyositis/ systemic Sclerosis)</td>
<td>3.4%</td>
</tr>
<tr>
<td>Jo-1</td>
<td>2.4%</td>
</tr>
<tr>
<td>CENP (centromeres)</td>
<td>2.9%</td>
</tr>
<tr>
<td>PCNA (proliferating cell nuclear antigen)</td>
<td>4.2%</td>
</tr>
<tr>
<td>ds DNA (Anti-double stranded DNA)</td>
<td>3.9%</td>
</tr>
<tr>
<td>Nucleosomes</td>
<td>3.4%</td>
</tr>
<tr>
<td>Histones</td>
<td>3.7%</td>
</tr>
<tr>
<td>Rib p protein (Antiribosomal P protein)</td>
<td>1.0%</td>
</tr>
<tr>
<td>AMA-M2 (Anti- mitochondrial m2 antibody)</td>
<td>3.9%</td>
</tr>
<tr>
<td>LC-1</td>
<td>3.2%</td>
</tr>
</tbody>
</table>

Discussion

ANA test is an initial test for diagnosing a suspected specific autoimmune disorder or ruling out other conditions with similar signs and symptoms. Patient with Systemic lupus erythematosus (SLE) is almost positive for ANA14,15. In our study, we estimated the frequency of test requests and the prevalence of ANA antigen along with the intensity of ANA. Patients with scleroderma or rheumatic diseases, or dermatomyositis have a positive ANA16. ANA testing can be used as a screening tool for specific symptoms of systemic lupus disease or other connective diseases17. Clinical and serological heterogeneity presents a significant diagnostic challenge, particularly at a very early stage. The criteria for the SLE classification are suggested by the American College of Rheumatology (ACR)18. In this study, the patient’s age group ranged from 1-86 years in ANA positive patients. The disease frequency was mostly between 20-60 years, with a female predominance. A study on Pakistani SLE patients showed that the mean age was 30.35±1.687 (12-68) years, and female to male ratio was 9.16:119. Agrawal et al. stated that 98% of patients were female, and 84% of patients were under 40 years of age in the Indian population14.

The predominance of women in the development of ANA means that female hormones and perhaps other factors play a significant role in autoimmune disease20,21. Several studies show age association with autoimmunity, which could be the cause for the ANA prevalence in the elderly. 22, 23, 24, 25, 26, 27. In this study, the prevalence of positive ANA test was observed in 27.8 % of patients. Kosaraju et al., (2010) reported ANA positive in 64.28 % and dsDNA in 89.36 % in 48 SLE patients. The female to male ratio was 15:113. A study by Minz et al.,(2012) stated that ANA positive was in 12.8% in 3453 suspected autoimmune disorders cases. The female to male ratio was 3:128.

The antinuclear antibodies and their subset have a role in the pathogenesis of the autoimmune disease.29 Antibodies against dsDNA, the serological hallmarks of SLE, were first described 60 years ago. The expression of antibodies to DNA associates with different clinical conditions30. SS-A (RO) Sjogren’s syndrome type ANA was found in the majority of the patients, followed by anti-nRNP (antinuclear ribonucleoprotein), anti-Sm (anti-Smith), anti-PCNA (anti-proliferating cell nuclear antigen), and anti-ds DNA (Anti-double stranded DNA). However, it is observed that only very few of these ANA have proven to be specific for SLE, such as anti nRNP, anti-Sm,anti-Rib-P, SS-A (RO), and to a lesser extent, anti-dsDNA antibodies30, 31. The presence of specific ANA in SLE is an important immunological marker for classifying the disease30. The majority of other antinuclear antibodies are found in rheumatic fever, systemic sclerosis, Sjogren’s syndrome, dermatomyositis, and many other autoimmune diseases30, 31.

Although ANA is a highly sensitive test used to screen suspected SLE patients, it cannot be considered a confirmation test for autoimmune disease due to its less specificity14. However, the results can support
the diagnosis of suspected SLE cases and monitor the disease prognosis. In addition to this, the pattern of subset test results could be used as a predictive marker and differential diagnosis of autoimmune disorders.

**Conclusion**

Substantial number of ANA tests were performed in suspected SLE patients to confirm the autoimmunity. The estimated prevalence of ANA positive tests was 27.8%, which differed in different sex and age groups. We identified that the SS-A (RO) Sjogren’s syndrome type A, ANA subsets were more prevalent, followed by nRNP(antinuclearribonucleoprotein), Sm (Smith), and PCNA (proliferating cell nuclear antigen) among the positive ANA cases. In autoimmune conditions, estimating the prevalence of ANAs influences the trends of antinuclear antibodies. The test frequency and its interpretation assist in patient diagnosis and management, along with specific diagnostic tests.

**Study Limitations:** This study provides retrospective data, and we could not correlate the ANA positive result with clinical and diagnostic features.

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**Author’s contribution**

Data gathering and idea owner of this study: Asha Patil, Kusumakshi Nayak, Chaithra, Deepa Kamath

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Editing and approval of final draft: Kusumakshi Nayak, Asha Patil.

**Reference**


9. Rao JS, Shobha V, Thomas T, Kini U. Standardizing initial dilution titers of antinuclear antibodies for
the screening of systemic lupus erythematosus.
https://doi.org/10.4103/injr.injr_172_18


