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The diagnostic utility of Ana, dsDNA and complement levels in patient with suspected autoimmune disease

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Abstract

The Antinuclear antibodies (ANA) and dsDNA are commonly used as a screening test for various suspected connective tissue disorders and autoimmune diseases. They help to monitor disease activity along with diagnostic and prognostic assessment. Systemic lupus erythematosus (SLE) is one of the main immune complex mediated disease. Antibodies to double-stranded deoxyribonucleic acid (dsDNA) are most sensitive for diagnosis of systemic lupus erythematosus. Infectious and environmental factors which, mainly include seasonal variation, interact for the autoimmune disease development. The seasonal variation found in ANA and dsDNA tend to increase during the colder months. Anti dsDNA in combination with the levels of complement proteins C3 and C4, fluctuate with changes in disease activity and are indicators of disease flare. Serum samples were obtained from Vardhman Mahavir Medical College and Safdarjung hospital. Serum samples were tested for the presence of ANA. The dsDNA and complement levels were done in the suspected SLE patients. The aim of this study was to find out the prevalence of ANA and anti dsDNA using Elisa in patient with suspected autoimmune disease along with the level of complement proteins by Nephelometry technique. Newer techniques like immune florescence can be used for better results.

Keywords: Systemic lupus erythematosus, ANA, Anti dsDNA, complement proteins

1. Introduction

Antinuclear antibodies are autoantibodies directed against cellular nuclear proteins.¹The ANA test is used as a screening tool for various connective tissue disorders and autoimmune diseases ^[2]. ANA and disease specific antibodies have also been used clinically to monitor disease activity along with diagnostic and prognostic assessment ^[3]. SLE is prototype of immune complex mediated disease. Antibodies to double-stranded deoxyribonucleic acid (dsDNA) are commonly used for diagnosis of systemic lupus erythematosus (SLE) ^[4]. The dsDNA show a disease correlation with patients suffering from SLE ^[5]. Infectious triggers, genetic background, immunological abnormalities and environmental factors interact for the autoimmune disease development. The seasonal variation is found in ANA and dsDNA preferably increased during the colder months possibly related to infections ^[6]. This seasonality is important in clinical practice as well. SLE is a chronic autoimmune disease characterized by relapses and flares with alternating periods of remission ^[7]. Anti dsDNA in combination with the levels of complement proteins C3 and C4, fluctuate with changes in disease activity. These are also one of the strong indicators of disease flare and treatment response in patients with lupus ^[8]. The decreased complement levels are eminent during flares of lupus activity and are believed to be secondary to increased autoantibody production and immune complex formation. The complement activation can also drive development of these pathogenic autoantibodies. Primary complement defects, especially in early components of the classical pathway, can lead to an increased susceptibility to SLE ^[9]. The aim of this study was to find out the prevalence of ANA and anti dsDNA in patient with suspected autoimmune disease along with the level of complement proteins.

2. Material and methods

Serum samples were obtained from Vardhman Mahavir Medical College and Safdarjung hospital for the study period lasted from December 2019 until December 2020. Each of these serum samples was tested for the presence of ANA (ANA screen elisa, Clabiotech, USA) and

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suspected SLE patients were tested for anti-dsDNA (dsDNA elisa, Clabiotech, USA) by ELISA method. The tests were performed by these commercial kits according to the manufacturer's instructions. Diluted patient serum is added to wells coated with purified nuclear antigens. IgG specific antibody binds to the antigen. All unbound material was washed away and enzyme conjugate was added to the antigen antibody complex. Excess enzyme conjugate was washed off and substrate was added. The plate was incubated to allow the hydrolysis of the substrate by the enzyme. An ELISA stopping solution was added to each well, and the plates were read at 450 nm. A dual wavelength is recommended with reference filter of 600-650nm. The intensity of the color was proportional to the amount of IgG specific antibody in the sample. Positive control, negative control and calibrators were run with each ELISA plate. Antibody index is calculated of each determination by dividing the optical density value of each samples by cut off value (calibrator O.D x calibrator factor). Nephelometry (Siemens) was used for complement levels which is based upon the principle that proteins form immune complex in an immunochemical reaction with specific antibodies. These complexes scatter a beam of light passed through the complex and this scattered light is proportional to the concentration of the relevant protein in the sample. (Normal range: c-3=80-160mg/dl; c4=20-40 mg/dl) The result is evaluate by comparison with a standard of known concentration. Positivity rates and Spearman correlation coefficient between assays were calculated as indicated using SPSS 21 software. In statistical analyzes, p-value <0.10 was considered as significant.

3. Results

In this study, we evaluated 1131 ANA and 31 anti-dsDNA results that were examined during one year period retrospectively in Lab medicine (Department of Pathology, Safdarjung hospital) from December 2018 to December 2019. A total of 58 (2.7%) sera were found ANA positive. ANA positivity rates in summer and winter were calculated as 3.4% AND 1.7% respectively. There were statistically significant differences for ANA positivity for winter and summer in study period. (p<.10) Eight (25.8%) patients were found positive for anti-dsDNA (Table 2). Anti-dsDNA positivity rates were calculated as 31.5% in summer and 16.6% in winter. Furthermore, anti-dsDNA results were not shown statistical significant difference in study period. Only one samples was found positive both ANA and anti-dsDNA. There is no statistically significant correlation between ANA and anti-dsDNA positivity (Figure 1, 2). Complement levels were done in 29 suspected SLE patients. Decreased level of c3 and c4 was found in 24.1 and 62% of patients respectively. (Table 3)

Table 1: ANA test results in study group

	Positive	Negative
Summer	22	607
Winter	09	493

Table 2: dsDNA test results in study group

	Positive	Negative
Summer	06	13
Winter	02	10

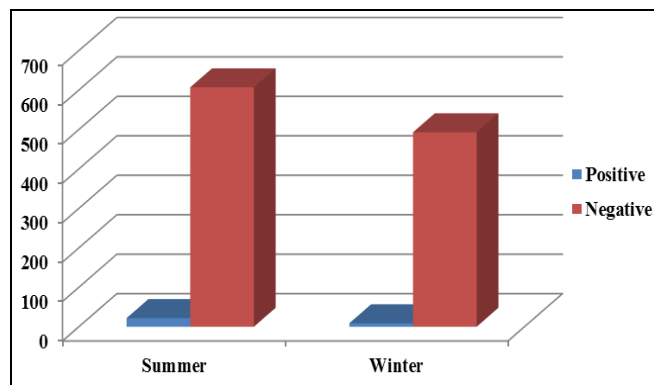


Fig 1: ANA test results comparison in study group

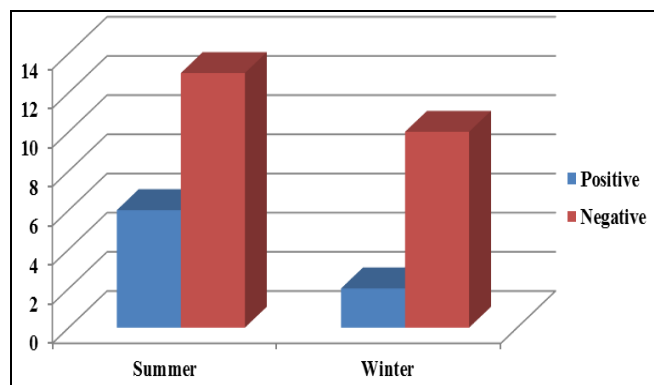


Fig 2: dsDNA test results comparison in study group

Table 3: Complement level test results in study group

	Normal	Decreased
C3	22	07
C4	11	18

4. Discussion

Circulating ANA are known to play a part in pathogenesis of autoimmune disease. IgG antibodies to dsDNA are considered clinically most useful for the diagnosis and management of SLE. The diagnosis of a patient with suspected autoimmune disease depends on the evaluation of different parameters -clinical findings, histopathology, tissue immunofluorescence, and serologic testing. ELISA is the method of choice for screening of anti dsdna in patient with suspected SLE [10]. ANA positivity rate in patient with suspected autoimmune disease varies as 8.7% to 34.4% in different part of world [11, 12]. In our study, results show that the ANA positive rate was 2.7% in patient with suspected autoimmune disease whereas dsDNA positivity rate was 25.8%. In the study conducted at Mangalore dsDNA positivity rate was 48% SLE patients. Both ANA and anti-dsDNA was done in 12 patient simultaneously. Positivity in both could be detected only in one patient, and other anti dsDNA positive patients were not shown ANA positivity simultaneously. With this study, ANA and anti-dsDNA data were evaluated for the first time in our region to the best of our knowledge. Additionally, anti dsDNA results were shown statistical significant difference in study period (p<0.05). Finally, in our study ANA and anti-dsDNA results has no correlation, Because of these major disadvantages for ELISA, ANA screen tests have to do with fluorescent technique. The fluorescent ANA test is a very good screening test for most of the previously discussed

antibodies 14, 15. Still, anti-dsDNA assessment can be conducted with ELISA for its higher sensitivity 16. Besides the investigation of monogenic forms of SLE over the years has triggered a better understanding of the SLE pathophysiological mechanisms. The findings that homozygous C1q deficiency and genetic mutations resulting in low levels of c2 and c4 significantly increase the risk of developing SLE are representative examples ^[9]. The ELISA and IIF results indicate that IgG and IgM ANAs are higher in SLE patients compared with normal patients. Interestingly, IgM anti-dsDNA antibodies negatively correlate with presence of lupus nephritis in SLE patients ¹⁷. These antibodies are useful in confirming the diagnosis in the clinical settings when SLE is likely to be the diagnosis. The main observation of our study was the aggravation of the disease activity in patients with SLE during the sunny season. It is noteworthy that the activation of SLE was mostly due to non-cutaneous reasons and was measurable. The results of previous investigations on seasonal variation in SLE activity are contradictory ^[18]. Exposure to sunlight is one of the environmental factors involved in the pathogenesis of systemic lupus erythematosus. Chiche L confirmed a seasonal pattern for lupus flares among patients living in Southern France, with most flares in spring, in correlation with an increase in temperature and duration of sunshine ^[19]. A similar seasonal pattern was observed in patients with no cutaneous involvement and with visceral involvement. The skin and joint activity are increased during the warmer months because of ultraviolet radiation exposure. Renal, sororities, and antids DNA levels are increased during the colder months, possibly related to infection. Similarly Collier and Levin reported a seasonal variation in anti dsDNA antibody levels with higher levels during the winter months ^[20]. With this study, ANA and anti-dsDNA data were evaluated for the first time in our region to the best of our knowledge. Additionally, anti dsDNA results were shown statistical significant difference in study period ($p < 0.05$). Finally, in our study ANA and anti-dsDNA results has no correlation, Because of these major disadvantages for ELISA, ANA screen tests have to do with fluorescent technique. Complement levels can help in intimation of flares in future. Hence these tests play an important role in diagnosis of autoimmune disease.

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