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Prevalence, Risk Factors and Clinico-Demographic Correlates of Antinuclear Antibodies in a Super speciality Hospital: Clinical Significance

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Abstract

For the diagnosis of autoimmune disorders detection of antinuclear antibodies (ANAs) is a key step. These are the hallmark of autoimmunity and are commonly seen in diseases like SLE, Scleroderma, polymyositis. Investigating the prevalence of ANA in patients suspected of autoimmune disorders and their clinical relevance. The clinico-demographic profile and risk factors present in the patient were correlated with ANA positivity. All samples received for ANA screening were tested for presence of ANA by ELISA. After that only patients with clinical symptoms suggestive of autoimmune diseases were tested for antibodies to ENA (extractable nuclear antigen) by LIA. Proforma was filled up for each patient to record the clinic-demographic details of the patients. Statistical analysis : All categorical data collected was analyzed using the GraphPad software. Descriptive analysis was done using percentage and association between variables were calculated using chi square test with significant P value < 0.05. Out of 2880, only 456 patients had signs and symptoms suggestive of autoimmune diseases and were included for further analysis.. Only 59 samples out of 456 were screened by both ELISA and LIA and were included in this study for further analysis of risk factors and clinico-demographic profile. Prevalence of ANA was found to be 7.8% (36/456). Female gender was significantly associated with ANA positivity (p=.0330). Joint pain and swelling was a significant clinical manifestation in patients who were ANA positive (p=.0375). Patients diagnosed with connective tissue disorders were significantly associated with ANA positivity as compared to infection/inflammation or other diagnosis (p=.0301). Most common antibody type among ANA positive cases found by LIA was SSA/Ro60 (50%) followed by SSA/Ro52 (22.2%) and PCNA (13.8%). DsDNA was present in connective tissue disorder while U1-SnRNP, SSB/La were present in infection and inflammation and other groups. Antibody types like Pm-Scl Sm-D1 and Ku were found in infection and inflammation. ANA positivity indicates towards autoimmune disease diagnosis especially CTDs with female gender being significant risk factor. ANA screening is commonly done for Arthritis. Patients presenting with fever, rash, joint pain, swelling, numbness and fatigue should be screened for ANA, followed by detection of antibodies against ENA which have more reliable diagnostic and prognostic roles.

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Keywords

SLE, Scleroderma, polymyositis, extractable nuclear antigen.

Introduction

Antinuclear antibodies (ANA) are the most common antibodies which include antibodies to both nuclear and cytoplasmic components in human cells (Meroni and Schur, 2010). These are the hallmark of autoimmunity and are commonly seen in diseases like SLE, Scleroderma, polymyositis etc. They are also present in organ-specific autoimmune diseases like autoimmune thyroiditis and hepatitis. In infections and cancer also ANA may be found (Tan, 1989; Satoh *et al.*, 2007; Tan *et al.*, 1997). Continuous efforts are being done by the researchers for diagnosing autoimmune diseases by developing ANA detection tests and specific antibodies to nuclear antigens (Kavanaugh *et al.*, 2000). However, a positive ANA test does not necessarily mean a disorder because 30% healthy individuals may be positive for ANA (Hu and Deng, 2014; Nagele *et al.*, 2013). A positive ANA test needs to be interpretated cautiously keeping the clinical presentation of the patient in mind. A positive ANA test is followed by detection of ENA (extractable nuclear antigen) antibodies to further differentiate between the different types of connective tissue disorders and other autoimmune diseases (Damoiseaux and Tervaert, 2006). This study was done with the aim of investigating the prevalence of ANA in patients suspected of autoimmune disorders and their clinical relevance. The clinico-demographic profile and risk factors present in the patient were correlated with ANA positivity.

Materials and Methods

This prospective study was done over a period of one year between January 2019 and December 2019 in the department of Microbiology of a super speciality hospital. The study was approved by the ethics committee of the institute.

Inclusion criteria

Serum sample of all patients with signs and symptoms suggestive of autoimmune disorder.

Exclusion criteria

Serum sample of all patients with non specific symptoms.

All samples received for ANA screening were tested for the presence of ANA by ELISA. Only patients with clinical symptoms suggestive of autoimmune diseases were tested again for antibodies to ENA(extractable nuclear antigen) by Line Immunoassay(LIA). Commercial kits (ANA screen ELISA by CALBIOTECH and IMTEC-ANA-LIA-MAXX by HUMAN, Germany.) were used and tests performed according to the manufacturer's instructions. A proforma was filled up for each patient to record the clinic-demographic details of the patients.

Statistical analysis

All categorical data collected was analyzed using the Graph Pad software. Descriptive analysis was done using percentage and association between variables were calculated using chi square test with significant P value < 0.05.

Results and Discussion

Total 2880 samples were received for ANA screening over one year period. Out of 2880, only 456 patients had signs and symptoms suggestive of autoimmune diseases and were included for further analysis. Rest all patients had non-specific symptoms like cough, headache, bodyache, less than 5 days fever, diarrhea, chest pain etc. for whom ANA testing was done but all of them were negative and excluded from analysis. Only 59 samples out of 456 were screened by both ELISA and LIA and were included in this study for further analysis of risk factors and clinico-demographic profile. The prevalence of ANA was found to be 7.8% (36/456). Out of 59, 36 samples were ANA positive and 23 ANA negative. The clinico-demographic profile was correlated between ANA positive and negative patients (table 1). Female gender was significantly associated with ANA positivity (p=.0330) with 72.2% females being ANA positive while only 27.7% males were ANA positive. Joint pain and swelling was a significant clinical manifestation in patients who were ANA positive (p=.0375). Patients diagnosed with connective tissue disorders were significantly associated with ANA positivity as compared to infection/inflammation or other diagnosis (p=.0301). No significant correlation was found between age and clinical manifestations with ANA positive status of the patient.

No significant risk factor was found to be associated with ANA positive cases (table 2). The most common antibody type among ANA positive cases found by LIA was SSA/Ro60 (50%) followed by SSA/Ro52 (22.2%) and PCNA (13.8%) (table 3). While among ANA negative cases, the most common antibody type was

SSA/Ro60 (26%) and Ku (26%). When the type of antibody was correlated with the clinical disease (table 4), it was found that SSA/Ro60, SSA/Ro52 and PCNA were found in all three clinical groups. Antibodies against dsDNA was present in connective tissue disorder while antibodies against U1-SnRNP, SSB/La were present in infection and inflammation and other groups. Antibodies against Pm-Scl Sm-D1 and Ku were found in infection and inflammation.

In the present study, overall ANA positivity rate was 7.8%. The presence of ANA among symptomatic patients suggests autoimmune disorder. ANA detection is in fact the serological hallmark of autoimmune disorder. The ANA positivity rate is less as compared to the study conducted by Minz *et al.,* (2012) in North India where ANA positivity was 12.3%. In another study by Thomas *et al.,* (2012) conducted in Netherlands, ANA positivity was 16.8% at tertiary care level. In another study conducted in Turkey [10] ANA positivity was 2.96%.

A positive ANA test always needs to be interpretated within the clinical context of the patient. It definitely helps the clinician to identify patients with autoimmune disorders but ANA testing is done for multiple diseases ranging from connective tissue disorders to infection /inflammation. And interpretation may be different for different diseases. In the present study clinico-demographic profile was correlated with ANA positivity.

It was found that female gender is significantly associated with ANA positivity(p=.0330). Other studies in India have also reported higher prevalence among females with female to male ratio 15:1 (Angel *et al.,* 2018; Kosaraju *et al.,* 2010; Paul *et al.,* 2003). Joint pain and swelling (arthritis) was the most significant clinical manifestation in ANA positive patients 27% (p=.0375).

This finding is similar to other studies where also the most common clinical manifestation was found to be arthritis (Kosaraju *et al.,* 2010; Paul *et al.,* 2003). Fatigue 52%, muscle pain 13%, weight loss and numbness 27% were some of the other clinical symptoms which were common but not statistically significant with ANA positivity status.

These symptoms (fatigue/muscle pain/weight loss) were also common in ANA negative patients (table 1) and maybe are non specific for autoimmune diseases. There are other non-autoimmune disorders also where these symptoms may be present. However, Angel *et al.,* (2018) reported skin rash and fever as most common clinical feature in patients with connective tissue disorders (CTD). The clinical diagnosis of patients was divided into three broad groups- connective tissue disorders that included SLE (systemic lupus erythmatosus), MCTD (mixed connective tissue disease), CREST SYNDROME, SS (Sjogren Syndrome) and PM (polymyositis); The infective/inflammatory group included RA (rheumatoid arthritis), Wegener's granulomatosis, Vascular diseases, Reynaud syndrome, Viral hepatitis, Autoimmune thyroid disease, Eye disorder, Primary biliary cirrhosis and Autoimmune hemolytic disease; Others included Cardiovascular disorder, Degenerative disorder of muscle, Leukemia, Lymphoma, Non-infectious hepatic disease, Malignancy, Diabetes and any other systemic disorder. In the present study it was observed that ANA positivity was more in patients diagnosed with CTD which was found to be statistically significant (p=.0301). Autoimmune response is seen in CTD where auto-antibodies are produced against nuclear self antigens like Smith, Ro52, SSA,SSB, Centromere, Scl70 etc. (Angel *et al.,* 2018; Mok and Lav, 2003; Manolios and Sehrieber, 1997). Several studies have shown that SLE is the most common CTD (Karakece *et al.,* 2015; Angel *et al.,* 2018; Kosaraju *et al.,* 2010). There was no significant correlation between age and ANA positivity in this study. However a study in U.S. reported that ANA positivity significantly increased with age (p<0.03) (Satoh *et al.,* 2012). Another study (Li *et al.,* 2011) showed similar findings as ours with ANA levels significantly higher in females and no significant association of ANA with age. So female gender is a risk factor for ANA positivity however no other risk factor was found to be significantly associated with ANA positivity. Identification of potential risk factors have a vital role in helping the clinicians in making presumptive diagnosis and starting therapy.

In ANA testing, for patients with suspected CTD/Autoimmune disorder, screening is done by ELISA for ANA and then a positive ANA is tested for DsDNA antibodies, antibodies against ENA (extractable nuclear antigens) like SSA, SSB, Sm,Scl-70, RNP depending on the clinical presentation (Agmon-levin *et al.,* 2014). The ENA are soluble in neutral buffers. Antibody against Sm antigen is highly specific for SLE. Anti U1RNP is seen in patients with SLE, systemic sclerosis and mixed CTDs and is also associated with myositis, oesophageal hypomotility, Raynaud's phenomenon, infrequent nephritis, arthralgias, arthritis, sclerodactyly and interstitial pneumonitis (Wanchu, 2000).

Table.1 Clinico-demographic profile correlation between ANA positive and ANA negative patients

Parameter	ANA positive N=36 (%)	ANA negative N = 23 (%)	P value
Age (yrs)			
0-10	0	0	1.000
11-20	1(2.7)	4(17.3)	.0704
21-30	9(25)	8(34.7)	.5569
31-40	15 (41.6)	4(17.3)	.0855
41-50	10(27.7)	3(13.04)	.2163
51-60	0	1(4.3)	.3898
>60	1(2.7)	3(13.04)	.2890
Gender			
Male	10 (27.7)	13(56.5)	.0330(S)
Female	26(72.2)	10(43.4)	
Clinical S/S			
Fatigue	19(52.7)	7(30.4)	.1127
Muscle weakness	5(13.8)	3(13.04)	1.000
Numbness /tingling sensation	10(27.7)	2(8.6)	.1026
Weight loss	10(27.7)	4(17.3)	.5376
Muscle pain	14(38.8)	7(30.4)	.5846
Dry eye/mouth	4(11.1)	3(13.04)	1.000
Skin rash	4(11.1)	1(4.3)	.6392
Hair loss	3(8.3)	3(13.04)	.6692
Joint pain/swelling	10(27.7)	1(4.3)	.0375(S)
Abdominal pain	3(8.3)	0	.2741
Recurring fever	5(13.8)	1(4.3)	.3886
Clinical diagnosis			
Connective tissue disorder	13(36.1)	2(8.6)	.0301(S)
Infection /inflammation	14(38.8)	14(60.8)	.1166
Others	9(25)	7(30.4)	.7628

Table.2 Correlation of Risk factors between ANA positive and ANA negative patients.

Risk factor	ANA positive N= 36	ANA negative N = 23	Odds ratio(95%CI)	P value
Antibiotic use	3	0	4.9104(0.2421 to 99.6011)	0.3001
Diabetes	5	1	3.5484(0.3871 to 32.5246)	0.2625
Hypertension	5	2	1.6935(0.3000 to 9.5612)	0.5508
Smoking	2	3	0.3922(0.0603 to 2.5509)	0.3272
Obesity	4	1	2.7500(0.2877 to 26.2898)	0.3798
Use of antiepileptic/ anti hypertensive drugs	3	0	4.9104(0.2421 to 99.6011)	0.3001
Family h/o of autoimmune disease	1	1	0.6286(0.0374 to 10.5739)	0.7471

Table.3 ENA Antibody profile of ANA positive and negative cases

	ANA Positive	ANA Negative
ENA type	Number N (%)	Number N (%)
PCNA	5 (13.8)	2 (8.6)
SSA/Ro60	18(50)	6(26)
SSA/Ro52	8(22.2)	0
SSb/La	3(8.3)	3(13)
Ku	2(5.5)	6(26)
RPP/Po	1(2.7)	0
Pm-Scl	1(2.7)	3(13)
Sm-D1	1(2.7)	3(13)
U1-SnRNP	2(5.5)	3(13)
dsDNA	2(15.3)	0

Table.4 Correlation of type of antibodies with clinical disease

Connective tissue disorder N=13(%)		Infection/inflammation N=14(%)		Others N=9(%)	
ENA type	Number	ENA type	Number	ENA Type	Number
PCNA	1(7.6)	PCNA	3(21.4)	PCNA	1(11.1)
SSA/Ro60	9(69.2)	SSA/Ro60	5(35.7)	SSA/Ro60	4(44.4)
SSA/Ro52	6(46.1)	SSb/La	2(14.2)	SSA/Ro52	2(22.2)
dsDNA	2(15.3)	Ku	2(14.2)	U1-SnRNP	1(11.1)
		RPP/Po	1(7.1)	SSB/La	1(11.1)
		Pm-Scl	1(7.1)		
		Sm-D1	1(7.1)		
		U1-SnRNP	1(7.1)		

Anti SSA(Ro) and Anti SSB(La) target two different ribonucleoprotein particles. Anti SSA(Ro) is associated with photosensitivity, lung disease, lymphopaenia and in some cases nephritis. Antibodiy against SSB(La) is associated with late-onset SLE, secondary SS, neonatal lupus erythematosus. (Wanchu, 2000; Castro and Gourley, 2010). Anti Pm-Scl are myositis specific antibodies (Castro and Gourley, 2010). The type of antibodies have a diagnostic and prognostic significance. The most common type of antibodies found were SSA/Ro60 (50%), SSA/Ro52 (22%) and PCNA (13.8%)in all three clinical groups. dsDNA antibody was found only in 15.3% CTD patient while other antibodies to U1-SnRNP and SSB/La, Ku were found in infection and inflammation group. Karakece *et al.,* (2015) found dsDNA in 4.52% patients with suspected autoimmune disorder. Anti-dsDNA antibodies are relatively specific for SLE. These appear in approximately 73% patients at some time or the other during the course of disease. Sometimes DsDNA is seen in normal persons, and individuals with SS and RA. In SLE their presence correlates with the presence of nephritis and disease activity (Wanchu, 2000). CTDs like SLE, systemic sclerosis, myositis, Sjogren's syndrome involve various organs and produce various auto-antibodies (Didier *et al.,* 2018). Some of these antibodies are just disease markers while some directly cause tissue damage. Knowledge of the type of auto-antibodies guides the clinicians in approaching to a diagnosis of CTDs (Bizzaro *et al.,* 2007). Angel *et al.,* (2018) in her study done in Tamil Nadu showed that common autoantibodies in CTDs like SLE, MCTD, Sjogren's syndrome were Sm, dsDNA, nRNP, SSA,SSB.; Scl-70 in scleroderma and SSA Ro52, nRNP, in polymyositis. Research has also shown that some auto-antibodies are associated with more than one disease like we found that SSA/Ro60, SSA/Ro52 and PCNA are present in all three types of clinical groups i.e. CTDs, infection/inflammation and other autoimmune disorders. Auto antibodies maybe detected in several viral infections like Hepatits A,B,C, Parvovirus B19, CMV, Enteroviruses etc. Viral infections induce autoimmune response via molecular

mimicry (Subuhi Sherwani *et al.,* 2018). In a study conducted in Brazil, SSA/Ro antibodies were the most common 41.7% followed by anti Scl-70 11.1% and anti RNP/Sm 8.3% and SSB/La 5.6% (Banhuk *et al.,* 2018).

The limitation of this study is that the patients were broadly divided into three clinical groups based on the clinical signs and symptoms and specific disease diagnosis was not correlated with ANA and ENA profile.

ANA detection may provide supporting evidence of autoimmune disease diagnosis especially CTDs. Female gender is a significant risk factor for ANA positivity. Arthritis being the most common clinical manifestation for which ANA screening is done. The most common age group affected is 31-40yrs old. Patients presenting with fever, rash, joint pain, swelling, numbness and fatigue should be screened by ANA, followed by detection of antibodies against ENA which have more reliable diagnostic and prognostic roles. Further research is needed to specially correlate clinical features with specific autoantibody profiles which was lacking in this study.

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