Original Research Article

Detection of prevalence of antinuclear antibodies in clinically suspected cases of systemic autoimmune diseases by indirect immunofluorescence test in a tertiary care hospital

Jabeen Begum[1*], V V Shailaja[1], Rajeshwar Rao[2]

[1*]Consultant Microbiologist, Department of Microbiology, AIG Hospital, Mindspace Rd, Gachibowli, Hyderabad-500032, Telangana, INDIA.
[1]Associate Professor, [2]Professor, Department of Microbiology, Gandhi Medical College, Secunderabad Telangana India 500003
Email: dr.jabeenbegum@gmail.com

Abstract

Background: Systemic autoimmune diseases (SAD) are the diseases where multiple organs are involved in the presence of auto antibodies directed against sub-cellular structures or molecules and are characterized by presence of Antinuclear antibodies (ANA). Indirect immunofluorescence test (IIFA) on Hep-2 (human epithelial cell tumour line) is a "gold standard" technique for detection of ANA **Purpose: 1)** To detect the Prevalence of Antinuclear antibodies (ANA) in clinically suspected cases of Systemic autoimmune diseases (SAD) by Indirect Immunofluorescence test(IIFA)on Hep2 cells **Methodology:** A total of 150 clinically suspected cases of SAD of both sexes and above 18yrs of age from various departments were included in the study and blood samples collected were subjected to Indirect Immunofluorescence test on Hep-2 cells coated slides and slides were visualized in fluorescent microscope using blue green filter (450nm). **Results:** 150 samples were analysed for ANA by IIFA. Out of 150 samples, 54 samples were positive by IIFA. Prevalence of ANA among clinically suspected case of SAD by IIFA was found to be 36%. Female predominance was seen and most common age group of presentation was 20-30yrs. The homogenous pattern was the most common (n=26; 48.14%) pattern followed by speckled pattern (n = 19; 35%). **Conclusions**: Systemic autoimmune disorders are chronic conditions with no cure and are growing day by day. With the increase in prevalence of systemic autoimmune diseases there is a need for early and accurate diagnostic techniques. Therefore, early detection of ANA by Gold Standard Indirect Immunofluorescence test in a Clinically suspected cases helps to reduce disease morbidity and mortality

Key Words: Systemic Autoimmune diseases, Antinuclear antibody test, Indirect immunofluorescence test.

[*]**Address for Correspondence:**
Dr.. Jabeen begum, Consultant Microbiologist, Department of Microbiology, AIG Hospital, Mindspace Rd, Gachibowli, Hyderabad, Telangana 500032, INDIA.
Email: dr.jabeenbegum@gmail.com

Received Date: 15/09/2018 Revised Date: 23/10/2018 Accepted Date: 09/11/2018
DOI: https://doi.org/10.26611/1008925

Access this article online	
Quick Response Code:	Website: www.medpulse.in
	Accessed Date: 24 February 2019

INTRODUCTION

Systemic autoimmune diseases are characterized by tissue injury due to breakdown of one or more of the basic mechanisms regulating immune tolerance leading to immunological reaction of the organism against its own tissues and are characterised by involvement of multiple organs with presence of a large variety of auto antibodies directed against sub-cellular structures or molecules. Diseases in this group includes Systemic lupus erythematosis (SLE), Systemic sclerosis/Scleroderma (SSc), undifferentiated connective tissue diseases or Mixed Connective tissue diseases (MCTD), Dermatomyositis / Polymyositis, Sjogren's syndrome (SS/SjS)[1]. Systemic autoimmune disorders are characterized by presence of Antinuclear antibodies (ANA) in the blood of patients. ANA are a specific class of autoantibodies that have the capability of binding and destroying certain structures within the nucleus of the

How to cite this article: Jabeen Begum, V V Shailaja, Rajeshwar Rao. Detection of prevalence of antinuclear antibodies in clinically suspected cases of systemic autoimmune diseases by indirect immunofluorescence test in a tertiary care hospital. *MedPulse International Journal of Microbiology*. February 2019;9(2): 23-27. https://www.medpulse.in/Microbiology/

cells and considered to be a serological hallmark of connective tissue diseases[2]. The prevalence of Systemic autoimmune diseases in India is increasing over years as it was 12.3% in 1996-2006, 18.9% in 2006-2007 as per retrospective North Indian study[3], 38.2% in 2010 from Vellore[4] and 35% in 2017 from Bhubaneswar[5]. This increase in prevalence is due to development and utilization of advanced diagnostic facilities for diagnosis of Systemic autoimmune diseases. ANA prevalence increases with age, with peak at 20–30 years of age and are more prevalent among females than males. No significant associations were seen with education, family income, alcohol use, smoking history[6]. The American College of Rheumatology (ACR) stated that ANA detection by IIFA on Hep-2 cells is considered as the gold standard[7]. In a Clinically suspected cases of connective tissue disorders, ANA test is done, if positive further tests are performed for the diagnosis of specific systemic autoimmune diseases. If negative no further autoantibody testing is performed[8,9].

MATERIALS AND METHODS

A cross-sectional study was conducted over a period of one and half year from March 2016 – Sept 2017 and 150 blood samples of Clinically suspected cases of Systemic autoimmune diseases of both sexes and more than 18yrs of age, were included in the study after informed consent.

Ethical Consideration: Ethical clearance was taken from institute's Ethical Committee. Detailed clinical and epidemiological and laboratory data were recorded using structured proforma. Under aseptic precautions 3ml of Blood samples was collected, Serum separated out and alliquoted. ANA detection by IIFA was performed on HEp-2000 cells according to the instructions provided by the manufacturer (Immuno Concepts, Sacramento, CA).

Procedure: Positive and Negative controls were run with each test daily. Serum was diluted in 1:80 ratio (serum: diluent) (10 µl serum +790 µl diluent). 30 µl of the diluted serum was then put on each wells. This was then incubated at room temperature for 30 min. This step allowed the antibodies in the serum to react with the antigens coated on the wells. The slide (wells) was then washed carefully and then dipped into the PBS for 10 min to remove the unbound antibodies. In the next step, FITC conjugate (Anti-human IgG conjugated to fluorescein isothiocyanate) was added to wells, to get bound to the antibodies and emit fluorescence. The FITC was again washed off carefully and dipped in PBS (in dark) for 10 min, to remove the unbound conjugate. The wells were then mounted using mounting medium. The visualization of the slide was then done under the fluorescence microscope at 40X. Based on the fluorescent intensity, samples were graded positive (+, ++, +++) and negative.

Negative: A serum was considered negative for antinuclear antibodies if nuclear staining was less than or equal to the negative control well with no clearly discernible pattern. The cytoplasm may demonstrate weak staining, with brighter staining of the non-chromosomal region of mitotic cells, but with no clearly discernible nuclear pattern.

Positive: A serum was considered positive if the nucleus shows a clearly discernible pattern of staining in a majority of the interphase cells. The positive sample showed bright apple green fluorescence in the nuclei of the cells, with a clearly discernible pattern characteristic of the control serum that was used.

Hep-2000 cells are transfected with the gene for SSA-60, which makes these cells more sensitive for SSA antibody detection. Serum samples were screened in a 1: 80 dilutions. FITC-conjugated goat anti-human IgG antibody was used for detection of ANA. Five staining patterns are commonly reported: homogenous, speckled, centromere, nucleolar and cytoplasmic. In caseof mixed-patterns, the pattern with the highest titre was included in the present study. Slides were evaluated with a fluorescent microscope (Axioskop, Carl Zeiss MicroscopyGmbH, Jena, Germany) with LED light source at 450nm using Blue- green

Statistical analysis: EPI INFO 7 version statistical soft ware package was used forstatistical analysis. Chi-Square test was applied to test whether differences between values were significant. p values <0.05 was considered as statistically significant andp value >0.05 as insignificant.

RESULTS

In the present study out of 150 samples from various departments majority of samples were from Rheumatology Department (43%), followed by Medicine Department (30.4%) and Dermatology Department (30%) as shown in table 1. The prevalence of ANA by IIFA was 36% as shown table 2. In the present study, majority of patients were found to be in the age group of 20yrs to 30yrs followed by 41yrs-50yrs. There was a significant association between age group 20-30years with ANA positivity (p value<0.05) as shown in table 3 and female preponderance was seen with female to male ratio of ANA positive cases were 6.5:1. as shown table- 4. Out of 54 ANA positive samples Homogenous is most common pattern on IIFA followed by Speckled. Various pattern of ANA on IIFA with their percentage prevalence is shown in table 5. Most common clinical manifestation was Joint pains seen in 122 cases (81.3%) followed by chronic low grade fever seen in 86 cases as shown in table 6. Among other laboratory investigation anaemia was most common finding, followed by elevated ESR as shown in table 7

Table 1: Department wise distribution of samples from clinically suspected cases of Systemic autoimmune diseases (SAD).

Departments	Total number (n=150)	ANA positive (n=53)	Percentage (%)
Rheumatology	79	34	43%
Dermatology	40	12	30%
Medicine	23	07	30.4%
Neurology	4	0	0%
Ophthalmology	4	0	0%
Surgery	2	0	0%
ENT	1	0	0%

Table 2: Detection of ANA by IIFA in Clinically suspected cases of SAD

Total no	ANA positive by IIFA	ANA negative by IIFA	Percentage Prevalence (%)
150	54	96	36%

Table 3: Age wise distribution of samples from clinically suspected cases of Systemic autoimmune diseases (SAD)

Age Limit	Total Number (n=150)	ANA Positive (n=53)	Percentage (%)
20-30 Year	97	45	**46.39%**
31-40 Year	19	3	15.7%
41-50 Year	30	5	16.66%
51-60 Year	4	0	0%

Table 4: Sex wise distribution of samples from clinically suspected cases of SAD

Sex	Number of clinically suspected Cases (n=150)	ANA Positive(n=53)
Female	105(70%)	46(86.7%)
Male	45(30%)	7(13.2%)

Table 5: Various Patterns of ANA on IIFA with their Prevalence

Patterns	Prevalence
Homogenous	26(48.14%)
Speckled	19(35%)
Speckled + Cytoplasmic	3(5.66%)
Cytoplasmic	2(3.77%)
Centromere	2(3.77%)
Nucleolar	1(1.88%)
Nucleolar + Homogenous	1(1.88%)

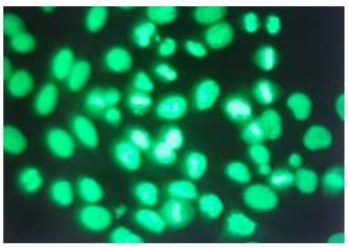

Figure 1

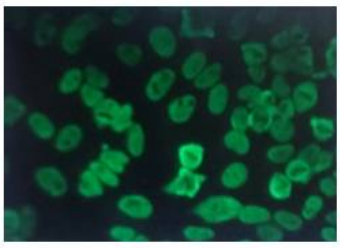

Figure 2

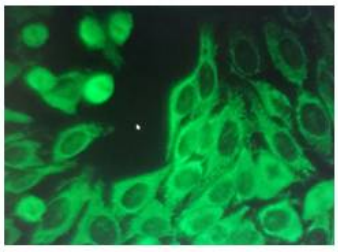

Figure 3

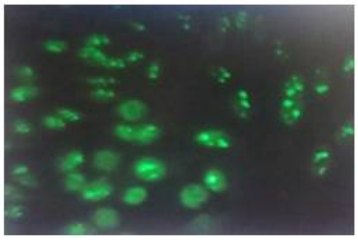

Figure 4:

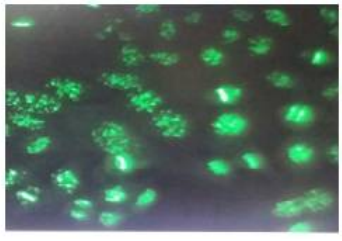

Figure 5:

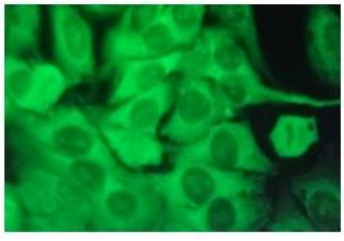

Figure 6:

Figure1: Homogenous pattern; **Figure 2:** Speckled pattern; **Figure 3:** Cytoplasmic+ Speckled pattern; **Figure 4:** Centromere pattern; **Figure 5:** Nucleolar pattern; **Figure 6:** Cytoplasmic pattern

How to cite this article: P K Patil, B S Patil. Pattern of anaerobic bacterial infection of diabetic foot. *MedPulse International Journal of Microbiology*. February 2019;9(2): 20-22. https://www.medpulse.in/Microbiology/

Table 6: Clinical manifestations in clinically suspected cases of SARD

Symptoms in clinically suspected cases of SARD	Number of cases (n=150)	Percentage (%)
Joint pains	122	81.30%
Chronic low grade fever	86	57.30%
Muscle Fatigue	62	41.30%
Weight Loss	42	28%
Rashes	36	24%
Hair Loss	33	22%
Photophobia	31	20.60%
Mouth ulcers	29	19.30%
Raynaud's phenomenon	26	17.30%

Table 7: Other Laboratory investigations in clinically suspected cases of SARD

Investigation	Number of Clinically suspected cases of SARD	Percentage (%)
Anaemia	44	29.30%
TLC < 4000/mcl	4	2.60%
PLATELETS <1.5lakhs/mcl	16	10.60%
ESR Raised	36	24%
RA Factor	14	9.30%

DISCUSSION

In the present study, Prevalence of ANA by IIFA was found to be 36% which was correlating with following other studies of Sarojini Raman *et al*, Shaily Garg *et al*, Wendy Sebastine *et al*, Akmatov *et al*, J. Angel Thomas *et al*. Most common Age group of presentation was 20-30yrs followed by 41-50yrs which is correlating with other studies of Sarojini Raman *et al*, Asaithambi *et al*, J. Angel Thomas *et al*,Mengeloglu. Z *et al*, Thomas Y. Avery *et al* and the probable reason can be due to physiologic stage of Reproductive age group and perimenopause implying that oestrogen may play an important role and genetic and hormonal factors may have an influence on disease manifestation. In the present study, clinically suspected cases of SAD is more predominant in females than in males (2.3:1) correlating with studies of (table-7) Sarojiniraman *et al*, M.E. Soto *et al*. The likely explanation for this female preponderance is probably related to exogenous and endogenous hormonal change, Homogenous pattern (48.14%) is most common pattern detected by IIFA correlating with studies of Wendy Sebastine *et al* (45.5%) from Vellore, J.Angel Thomas *et al* (44%) from Tamil Nadu, Asaithambi *et al* (44.4%) from Tamil Nadu, Thomas Y. Avery *et al* (40%) from Netherland, Sarojiniraman *et al* (52.6%)from Bhubaneswar. Homogenous pattern mostly associated with autoantibodies to dsDNA which is diagnostic criteria for SLE. Hence indirectly indicating that SLE is the most common systemic autoimmune disease reported

CONCLUSION

Systemic autoimmune disorders are chronic conditions with no cure and with the increase in prevalence of systemic autoimmune diseases there is a need for early and accurate diagnostic techniques. Ther+efore, early detection of ANA by Gold Standard Indirect Immunofluorescence test in a Clinically suspected cases helps to reduce disease morbidity and mortality

ACKNOWLEDGEMENT

The authors are thankful to Dr. K. Nagamani Professor and Head of Department of Microbiology, to faculty, technicians, non-teaching staff of Department of Microbiology, Gandhi Medical College, Secunderabad, Telangana, India, for providing facilities to carry out the research work. We are also thankful to Medicine, Rheumatology and SPM departments for their support to carry out this work.

REFERENCES

1. Jacinth Angel, Marin Thomas, BoppeAppalaraju Department of Microbiology, PSG Institute of Medical Sciences and Research, Coimbatore, Tamil Nadu, India Evaluation of ELISA and indirect Immunofluorescence in the diagnosis of autoimmune diseases and their interpretation in the clinical situation J AcadClinMicrobiol 2015 [cited 2017 Aug 6];17:7-11. DOI: 10.4103/0972-1282.158776
2. MinzRW, KumarY, An and S,etal.Antinuclear antibody positive autoimmune disorders in NorthIndia :an appraisal. Rheumatol Int. 2012; 32:2883–2888.
3. Wendy Sebastian, Atanu Roy1, Usha Kini1, Shalini Mullick1 Correlation of antinuclear antibody immunofluorescence patterns with immune profile using line immunoassay in the Indian scenario Indian Journal Of Pathology And Microbiology - 53(3), July-September 2010 DOI: 10.4103/0377-4929.68262
4. Sarojini Raman, Amit Kumar Adhya, Prasantkumar Pradhan, Kanaklata Dash, Urmila Senapati: Correlation of Indirect Immunofluorescence and Line Immunoassay Method in Detection of Autoimmune Diseases: an

Observational Study at a Tertiary Care Teaching Hospital Sch. J. App. Med. Sci., 2017; 5(7A):2520-252
5. SatohM, ChanEK, HoLA, etal. Prevalence and sociodemographic correlate so antinuclear antibodies in the United States. Arthritis Rheum. 2012;64: 2319–2327.
6. J. G. M. C. Damoiseaux and J.W. Cohen Tervaert, "FromANAto ENA: how to proceed?" Autoimmunity Reviews, vol. 5, no. 1,pp. 10–17, 2006
7. F. Alvarez, P. A. Berg, F. B. Bianchi *et al*., "International autoimmune hepatitis group report: review of criteria for diagnosis of autoimmune hepatitis," Journal of Hepatology, vol. 31, no. 5, pp. 929–938, 1999..
8. A. F. Kavanaugh, D. H. Solomon, and The American College of Rheumatology ad hoc Committee on Immunologic Testing Guidelines, "Guidelines for immunologic laboratory testing in the rheumatic diseases: anti-DNA antibody tests," Arthritis Care and Research, vol. 47, no. 5, pp. 546–555, 2002
9. Shaily Garg, Anshika Srivastava* and Santosh Prasad: Correlation of Line Immuno Assay with Indirect Immunofluoresence Assay for the Detection of Anti-Nuclear Antibodies in Various Autoimmune Disorders ; Journal of Autoimmune Disorders ISSN 2471-8513 Vol.3 No.3:37 2017
10. ManasAkmatov, NadjaRober, Wolfgang Ahrens, Dieter Flesch-Janys,JuliaFRicke,HalinaGresiser,KathrinGunther,Rudolf kaaks,YyonneKemmling,BastianKrone,JakobLinseisen,C hristaMeisinger,SusanneMoebus, Nadia Obi, Carlos A.Guzman,Karsten Conrad and Frank Pessler. Arthritis Research and Therapy (2017) 19:127 DOI 10.1186/s13075-017-1338-5
11. Zafer Mengeloglu1, TekinTas, EsraKocoglu, GülaliAktas, SeydaKarabörk Determination of anti-nuclear antibody pattern distribution and clinical relationship Pak J Med Sci 2014 Vol. 30 No. 2
12. Thomas Y. Avery, MartvandeCruys, JosAusten, FransStals, and JanG. M.C. Damoiseaux Anti-Nuclear Antibodies in Daily Clinical Practice: Prevalence in Primary, Secondary, and Tertiary Care Journal of Immunology Research Volume 2014, Article ID 401739, 8 pages. doi.org/10.1155/2014/401739
13. I Peene, L Meheus, E M Veys, F De Keyser Detection and identification of antinuclear antibodies (ANA) in a large and consecutive cohort of serum samples referred for ANA testing Ann Rheum Dis 2001;60:1131–1136
14. María Elena Soto, Nidia Hernández-Becerril, Ada Claudia Perez-Chiney, Alfredo Hernández-Rizo, José Eduardo Telich-Tarriba, Luis Eduardo Juárez-Orozco,Gabriela Melendez, and Rafael Bojalil·Predictive value of antinuclear antibodies in autoimmune diseases classified by clinical criteria: Analytical study in a specialized health institute, one year follow-up. Results Immunol. 2015; 5: 13–2
15. AnupriyaAsaithambi, ManjulaGunasekaran, Manivelan S, PalaniappanNainar Antinuclear Antibodies in Patients with unexplained recurrent abortions Asian J Pharm Clin Res, Vol 10, Issue 8, 2017, 256-259
16. Ya-Ping Guo, BS, Chun-GuangWang,MD, XinLIU,MS,Yi-Qian Huang, BS,De-Li Guo,BS, Xing-ZhuoJing,BS, Chun-Gang Yuan,PhD,Song Yang, BS, Jin-Mei Liu,BS, Meng-Si Han, BS, Hong-Xing Li,PhD..The Prevalence of Antinuclear Antibodies in the General population of China : a Cross-Sectional Study Current Therapeutic Research 76 (2014) 116–119
17. PriyadarshiniShanmugam, JeyaMeenakshisundaram AndPothini J A Comparative Study Of Enzyme Linked Immunosorbent Assay (Elisa) With Immunofluorescence Assay (Ifa) For The Detection Of Anti Nuclear Antibodies International Journal of Pharma and Bio Sciences Int J Pharm Bio Sci 2013 Oct; 4(4): (B) 75 – 80.

Source of Support: None Declared
Conflict of Interest: None Declared

How to cite this article: P K Patil, B S Patil. Pattern of anaerobic bacterial infection of diabetic foot. *MedPulse International Journal of Microbiology*. February 2019;9(2): 20-22. https://www.medpulse.in/Microbiology/