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PATTERN OF ANTI-U1-SN RNP ANTIBODY DISTRIBUTION USING LINE IMMUNOASSAY (LIA) PLATFORM IN PATIENTS' ATTENDING A TERTIARY CARE HOSPITAL IN BIHAR

Dr. Neelam Kumari[1], Dr. Mala Mahto[2], Dr. Ayan Banerjee[3], Dr. Sushil Kumar[4], Dr. Mukunda[5], Dr. Rajeev Ranjan[6]

[1]Senior Resident, Department of Biochemistry, All India Institute of Medical Science, Patna, Bihar, India.
[2]Associate Professor, Department of Biochemistry, All India Institute of Medical Science, Patna, Bihar, India.
[3]Associate Professor, Department of Biochemistry, All India Institute of Medical Science, Patna, Bihar, India.
[4]Associate Professor, Department of Biochemistry, All India Institute of Medical Science, Patna, Bihar, India.
[5]Senior Resident, Department of Biochemistry, All India Institute of Medical Science, Patna, Bihar, India.
[6]Tutor, Department of Microbiology, Vims, Pawapuri, Nalanda, Bihar, India.

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Corresponding author: Dr. Neelam Kumari
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Abstract

Aims and Objective: To observe the age and sex wise distribution pattern of anti-U1snRNP antibody in patients attending AIIMS Patna using Line immunoassay platform (LIA), study the pattern of anti-U1sn RNP antibody positivity depending on intensity of bands observed and study the association of anti-U1snRNP antibody positivity with other antibodies comprising the ANA profile.

Material and Methods: A retrospective study on all samples received for ANA profile was done using line immunoassay (LIA) in Biochemistry central lab spanning the period from January 2019 to January 2020.Approximately 1200-1300 samples received for antibodies against nuclear antigens during this period were analysed for positivity of anti-U1snRNP antibody.Patient's result data was categorised age and sex wise. The positivity of anti-U1snRNP will be graded as equivocal,(+),(++),(+++) depending on intensity of band with reference to cut off control. The anti-U1snRNP positivity will be recorded as single positivity or positivity along with other antibodies comprising the ANA profile.

Results: A total of 176 U1-SnRNP antibody positive patients were identified,1148 sera tested by LIA in which 588 samples were positive against 17 autoantigens (dsDNA,nucleosome,histone,SmD1,PCNA,P0,SS-A/Ro52,SS-A/Ro60,SS- B/La,CENP-B,Scl-70,U1-snRNP,AMA-M2,Jo-1,PM-Scl,Mi-2 and Ku).89 samples were identified for equivocal. So here we analysed only 255 samples,176 for U1-SnRNP positivity and 89 for equivocal. Sex distribution of 176 U1-SnRNP positive samples are showing in graph 1, 71% females and 29% males are positive against U1-SnRNP. we found that 77 females are positive for +1 and 18 for +2 while 30 for +3 grade which is significant.40 males +1,8 for +2 and 3 males showing+3 grade positivity. We found that 35.20% patients are between 25-40 years of age, 18.80% for pediatric age group,16.50% patients in15-24 yrs while 29.50% patients are above the 40 years of age.

Conclusion: Pattern of anti-U1-sn RNP antibody distribution using line immunoassay (LIA)as early markers for diagnosis of diseases.

Introduction:

Autoimmune diseases are a manifestation of a self-damaging immune response of the body to its own antigen[1]. The systemic rheumatic diseases rarely have a single pathognomonic diagnostic criterion due to involvement ofmultiple organs and system. Interestingly, each member of this disease group may be associated with different auto -antibody types[2]. The diagnosis depends on the clinical features as well as the presence of auto-antibodies in the serum. These antibodies target the specific antigen in the nuclear part of the cell although at times they can also react with other cellular organelle like cytoplasm, mitochondria, nuclear membrane etc[3]. The presence of these auto-antibodies in sera provide important clue regarding diagnosis and prognosis of disease. Many a times the presence of an auto antibody precedes the onset of clinical manifestations. They are used to monitor response to treatment also. Traditionally, ANA is measured most commonly by two ways: Indirect immune fluorescence which can report positivity along with pattern identification or negativity. The other format of reporting ANA involves quantification of specific auto-antibodies.ANA /ENA profile is a commonly done test aiming at detection of multiple auto-antibody against many nuclear self-antigens[4] .Antibody to U1-snRNP is one such antibody which targets the U1 small nuclear ribonucleoprotein particle(snRNP) .The U1-sn RNP is a

target of autoreactive B cells and T cells in several rheumatic diseases including SLE and Mixed connective tissue disease(MCTD).Mixed Connective tissue disease (a type of autoimmune rheumatic disease) was originally defined in 1972 as a connective tissue disorder characterised by the presence of high titers of a distinctive autoantibody, now called the anti-U1 ribonucleoprotein. The central premise of the MCTD concept is the presence of anti-U1sn RNP antibodies along with anoverlap of clinical features suggestive of SLE, Systemic sclerosis, idiopathic inflammatory myositis and rheumatoid arthritis. Through this study we intend to focus on the pattern of anti-U1sn RNP distribution in patients attending AIIMS Patna for symptoms suggestive of connective tissue diseases[5-7].

Materials and Methods

A retrospective study on all samples received for ANA profile was done by Line immunoassay (LIA) in Biochemistry central lab spanning the period from January 2019 till January 2020.Approximately 1200-1300 samples received for antibodies against nuclear antigens during this period were analysed for positivity of anti-U1snRNP antibody. This **research has approved by institutional ethical committee of AIIMS, Patna, Bihar**. Patient's result data was categorised age and sex wise. The positivity of anti-U1snRNP were graded as equivocal,(+),(++),(+++) depending on intensity of band with reference to cut off control. The anti-U1snRNP positivity was recorded as single positivity or positivity along with other antibodies comprising the ANA profile.

Inclusion criteria:

1. All samples received in Biochemistry central lab for ANA profile and subsequent anti-U1snRNP positivity.

Exclusion criteria:

1. All lipaemic, icteric and haemolysed sample were rejected.

Venous blood collected under aseptic conditions in vaccutainers without anticoagulant and put to centrifugation for serum separation. The separated serum and stored in -20 degree refrigerator in mini centrifuge vials till analysis.

The sample will be processed by Line immunoassay (LIA)for detection of anti-nuclear antibody panel which includes antibodies to 17 antigens (dsDNA, nucleosome, histone, SmD1,PCNA,P0,SS-A/Ro52,SS-A/Ro60,SS- B/La, CENP-B,Scl-70,U1-snRNP,AMA-M2,Jo-1,PM-Scl,Mi-2 and Ku).LIA is an indirect membrane based enzyme immunoassay for the qualitative measurement of IgG class antibodies against the above mentioned seventeen nuclear antigens in human serum or plasma. The kits used are provided by Human Diagnostics (IMTEC-ANA-LIA MAXX) and the instrument used is semi-automated analyser OZOBLOT 40M provided by Medsource Ozone Biomedicals.

Principle of the test:

The test is based on the principle of line immunoassay.Nuclear and associated cytosolic antigens are applied as lines on a nitrocellulose membrane.The nitrocellulose membrane are blocked to prevent unspecific reactions. During incubation of astrip with diluted patient's samples autoantibodies present in the sample will bind to the antigens on the strip.For the detection of the bound antibodies a secondary horse radish peroxidase (HRP)-labelled anti-human IgG antibody is used. After addition of the substrate and stop solution the appearance of brown lines indicate the existence of (auto) antibodies against the respective antigens.

The interpretation of the test results takes place exclusively on the basis of the respective cut-off control regarded for each strip:

a)The test result is negative if no band is to be recognised or if the band exhibits a smaller intensity in comparison to the cut -off control.

b) The test is equivocal if the intensity of the band and the intensity of the cut off control do not significantly differ.

c) The test result is positive, if a band exhibits a stronger staining in comparison to the cut-off control.

Statistical analysis

The recorded data was compiled entered in a spreadsheet computer program (Microsoft Excel 2010) and then exported to data editor page of SPSS version 20 (SPSS Inc., Chicago, Illinois, USA). Descriptive statistics included computation of percentages, means and standard deviations. Test applied for analysis was chi-square test. The confidence interval and p-value were set at 95% and 5%.

Results

A total of 176 U1-SnRNP antibody positive patients were identified,1148 sera tested by LIA in which 588 samples were positive against 17 autoantigens (dsDNA, nucleosome,histone,SmD1,PCNA,P0,SS-A/Ro52,SS-A/Ro60,SS- B/La,CENP-B,Scl-70,U1-snRNP,AMA-M2,Jo-1,PM-Scl,Mi-2 and Ku).89 samples were identified for equivocal. So here we analysed only 255 samples,176 for U1-SnRNP positivity and 89 for equivocal. Sex distribution of 176 U1-SnRNP positive samples are showing in graph 1, 71% females and 29% males are positive against U1-SnRNP.

Table 1: Sex distribution with pattern of U1-SnRNP

Gender	Grades of UI- SnRNP positivity			P value (chi square test)
	+	++	+++	
	n (%)	n (%)	n (%)	
Female	77(61.60)	18(14.40)	30(24.0)	< 0.01
Male	40(78.43)	8(15.69)	3(5.88)	
Total	117(66.48)	26(14.77)	33(18.75)	

Test applied: chi-square test

Table 2: Showing age with pattern of U1-SnRNP positivity.

	UI-SnRNP Positivity				p-value
AGE (years)	+	++	+++	Total	
	N (%)	N (%)	N (%)		
<14	27(81.82)	3(9.09)	(9.09)	33(100)	<0.05
15-24	12(41.38)	7(24.14)	10(34.48)	29(100)	
25-40	43(70.49)	10(16.39)	9(14.50)	62(100)	
>40	32(65.31)	7(18.50)	13(25)	52(100)	

Test applied: chi-square test

Table 3: Showing the association between U1-SnRNP positivity and Nucleosomes positivity

U1-SnRNP Positivity	Nucleosomes			p-value
	Absent	Present		
	N (%)	N (%)	Total	
+	113(96.58)	4(3.42)	117	<0.05
++	24(92.31)	2(7.69)	26	
+++	20(84.85)	5(15.15)	33	
Total	165(95.65)	11(6.25)	176.0(100.0)	

Test applied: chi-square test

Table 4: Association between U1-SnRNP positivity and SmD1 positivity.

U1-SnRNP Positivity	SmD1			p-value
	Absent	Present		
	N (%)	N (%)	Total	
+	104(88.89)	13(11.11)	117	
++	24(92.31)	2(7.69)	26	<0.001
+++	21(63.64)	12(36.36)	33	
Total	149(84.66)	27(15.34)	176.0(100.0)	

Test applied: chi-square test

Table 5: Association between U1-SnRNP positivity and SS-A/RO60 positivity.

U1-SnRNP Positivity	SS-A/RO60			p-value
	Absent	Present		
	N (%)	N (%)	Total	
+	103(88.03)	14(11.97)	117	
++	23(88.46)	3(11.54)	26	<0.001
+++	16(48.48)	17(51.52)	33	
Total	142(80.68)	34(19.32)	176.0(100.0)	

Test applied: chi-square test

Table 6: Association between U1-SnRNP positivity and SS-A/RO52 positivity.

U1-SnRNP Positivity	SS-A/RO52			p-value
	Absent N (%)	Present N (%)	Total	
+	114(97.44)	3(2.56)	117	<0.001
++	24(92.31)	2(7.69)	26	
+++	23(69.70)	10(30.30)	33	
Total	161(91.48)	15(8.52)	176.0(100.0)	

Test applied: chi-square test

Table 7: Association between U1-SnRNP positivity and Ku positivity.

U1-SnRNP Positivity	Ku			P value (chi square test)
	Absent N (%)	Present N (%)	Present N (%) Total	
+	102(87.18)	15(12.82)	117	<0.05
++	23(88.46)	3(11.54)	26	
+++	29(87.88)	4(12.12)	33	
Total	154(87.50)	22(12.50)	176.0(100.0)	

Test applied: chi-square test

Table 8: Association between U1-SnRNP positivity and SS-B/La. positivity.

U1-SnRNP Positivity	SS-B/La			p-value
	Absent N (%)	Present N (%)	Total	
+	101(86.32)	16(13.68)	117	<0.01
++	23(88.46)	3(11.54)	26	
+++	21(63.64)	12(36.36)	33	
Total	145(82.39)	31(17.61)	176.0(100.0)	

Test applied: chi-square test

Table 9: Association between U1-SnRNP positivity and Mi-2. positivity.

U1-SnRNP Positivity	Mi-2			p-value
	Absent N (%)	Present N (%)	Total	
+	109(93.16)	8(6.14)	117	<0.05
++	20(76.92)	6(23.08)	26	
+++	31(93.94)	2(6.06)	33	
Total	160(90.91)	16(9.09)	176.0(100.0)	

Test applied: chi-square test

Discussion

In agreement with the general theme of these findings, it has been reported that there are more than 20 autoantibodies that have a role in etiology of SLE [8]. Anti-dsDNA antibody is the first autoantibody that has been certified as a clinical diagnostic marker for SLE [9], and the present results support such diagnostic importance irrespective of the clinical subtypes of SLE; arthritis and nephritis, as both groups showed an increased percentage of positive cases , and without significant difference between them. Anti-dsDNA antibodies have also been detected in certain SLE patients prior to the onset of

nephritis as measured by severe proteinuria. However, a retrospective study concluded that anti-dsDNA antibodies are not predictive of renal flare in SLE patients, but they have a strong sensitivity (80%) for severe SELENA-SLEDAI flare in patients [10]. It has also been suggested that these antibodies may participate in initiating SLE nephritis, and SLE has been proposed to be a prototype of immune complex nephritis in human [11].

In our study total of 176 U1-SnRNP antibody positive patients were identified,1148 sera tested by LIA in which 588 samples were positive against 17 autoantigens (dsDNA,nucleosome,histone,SmD1,PCNA,P0,SS-A/Ro52,SS-A/Ro60,SS- B/La,CENP-B,Scl-70,U1-snRNP,AMA-M2,Jo-1,PM-Scl,Mi-2 and Ku).89 samples were identified for equivocal. So here we analysed only 255 samples,176 for U1-SnRNP positivity and 89 for equivocal. Similar results was found by Sood et. Al from North India screened 1000 patients with connective tissue diseases including RA attending a tertiary care centre and detected only three patients with MCTD[12]. Nedumaran et al from South India described eight patients of MCTD among 6400 patients with rheumatic disease [13]. Because all diagnostic criteria developed so far rely on presence of anti-U1sn RNP antibody in high titers and assays for detection of anti-U1snRNP may have limited availability across India, many cases of MCTD may be missed out across India.

in our study, 71% females and 29% males are positive against U1-SnRN.female were predominant in our study .similar finding in other study such as 21 et al they found predominantly a disease of females as is the case with majority of the autoimmune diseases,with a female to male ratio of 16:1[14].

In our study the sex distribution with pattern of U1-SnRNP,here we found that 77 females are positive for +1 and 18 for +2 while 30 for +3 grade which is significant.40 males +1,8 for +2 and 3 males showing+3 grade positivity.

In our study we found that 35.20% patients are between 25-40 years of age,18.80% for pediatric age group ,16.50% patients in15-24 yrs while 29.50% patients are above the 40 years of age.Nakae K et al similar age groups range from 4-80 years, the mean age of onset in adults being 35 years and 10 years in children was found . The incidence is low when compared with other connective tissue diseases like SLE, Dermatomyositis, polymyositis, rheumatoid arthritis and scleroderma[14].

The association of U1-SnRNP with 16 antigens ,or the prevalence of antigen 19.32% in our study in other study and similar result were found in other studies.The prevalence of anti-RNP autoantibodies varies depending on the disease. Anti-RNP autoantibodies are detected in 30–40% of SLE patients and in nearly all MCTD patients, as anti-RNP reactivity is a criteria for the diagnosis of CTD[15-17].Koszarny et al. also documented the positivity of SLE patient's sera for such autoantibody but at a lower prevalence (13.3%) in Polish SLE patients especially those that had arthritis[18]. It has been suggested that production of this autoantibody in sera of patients with autoimmune diseases can result from the exposure to some chemical xenobiotics compounds such 2- nonynoic acid, in addition, to the cross reactivity due to molecular mimicry of some infectious agents (for instance E. coli) [19].

In our study the association between U1-SnRNP positivity and SmD1 positivity was (Pearson chi2(2)=14.0126 ,pr=0.001). Hu C et al found the high positivity of anti-SmD1 antibody in Chinese SLE patients; 68% in non-treated SLE and 58.8% in treated SLE patients [20]. The seropositive prevalence of anti-SmD1 antibody was 55.6% in SLE patients, 44.8% in arthritis patients, and 39.7% in renal disorder [21]. Anti-SmD1 antibody is directed against many epitopes that found in the core of several proteins. These epitopes induce T-cell response, and Epstein Barr virus (EBV) is one of these epitopes that has a similar self-amino acids sequence, leading to a reaction between anti-SmD1 antibody and these epitopes, and then stimulating T-lymphocyte immune response cascade[22].

In our study the association between U1-SnRNP positivity and SS-A/RO60 positivity was (Pearson chi2(2)=27.0159 pr=0.000),the association between U1-SnRNP positivity and SS-A/RO52 positivity (Pearson chi2(2)=25.4306 pr=0.000) and the association between U1-SnRNP positivity and Ku positivity was (Pearson chi2(4)=9.7072 pr=0.046).

Mannik et al. detected a number of autoantibodies in the sera of nephritis SLE patients; anti- nucleosome, anti-smD1, anti-SS-A/Ro and anti-SS-B/La; however, they did not prove that these autoantibodies may have a role in the development of SLE, but it has a role in causing inflammation [23]. These autoantibodies may arise after apoptosis of inflamed kidney cells. Whereas, Gronhagen and Nyberg and Fu et al. referred to the role of anti-histone, anti-nucleosome and anti-SS-A/Ro in cutaneous lupus, and these auto-antibodies were associated with an increased risk of photosensitive rash development [24,25]. However, there have been no recorded results about the role of these autoantibodies in arthritis SLE.

In a previous study, the presented frequency of this autoantibody in the circulation of Kuwaiti SLE patients was 13% with the presence of anti-ds-DNA (35.5%), anti-ANA (96.8%), anti-SmD1 (13%), anti-RNP (13%), anti-SS-A/Ro (35.5%), and anti-SS-B/La (19.4%) autoantibodies[26]. The increased level of this autoantibody may risk the patient for pulmonary fibrosis, diffuse cutaneous disease and nephritis [27].In our study the association between U1-SnRNP positivity and SS-B/La. positivity was (Pearson chi2(2)=9.9068 pr=0.007).VenrooijWJ et al found same

result with antibodies against the RNA component of U1-snRNP are found in 38% of anti-RNP positive patient sera[28]. In one study, anti-U1-A antibodies were detected in greater than 90% of antiU1-snRNP sera from patients with rheumatic disease[28]. Most MCTD patients have high anti-U1snRNP but anti-U1 snRNP is not exclusive to MCTD(1).Anti-U1snRNP seemed to be a robust marker of MCTDonset. Its emergence preceded the onset of clinical manifestations[29].

Conclusion

Pattern of anti-U1-sn RNP antibody distribution using line immunoassay (LIA)as early markers for diagnosis of diseases

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