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THE PREVALENCE OF EXTRACTABLE NUCLEAR ANTIGEN ANTIBODIES IN THE GENERAL POPULATION OF PAKISTAN A RETROSPECTIVE STUDY

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Abstract:

OBJECTIVE: *To determine the prevalence of extractable nuclear antigen antibodies in general population of Pakistan*

Study Design: *Retrospective study*

Place and Duration*: Department of chemical pathology and immunology Chughtai Lab Lahore from January 2018 to December 2018.*

Patients and Methods: *Data was retrieved from Nexus pro, laboratory information management system installed at Chughtai lab Lahore after approval from research ethics committee. Data of twenty five (25) anti ENA antibodies of 1268 subjects were collected and entered in SPSS version 23.0 for analysis. The samples of these patient were analyzed on D-Tek blue dot driver instrument which is based on the principle of immunoblot.*

RESULTS. *The mean age of the study population was 38.08±15.38 years. Females patients were 675(67%) which were higher than males 325(32%). The frequency of all twenty five antibodies were determined which are as follows: Anti Nucleosome IgG56(5.6%), Anti dsDNA IgG76(7/6%), Anti Histones72(7.2%), Anti Sm IgG63(6.3%). Anti-Ribosome PO IgG15(1.5%), Anti PCNA IgG, 52(5.2%), Anti RNP IgG 68kD/A/C88(8.8%), Anti Sm/RNP IgG124(12.4%), Anti SSA(Ro) IgG 60kD173(17.3%), SSA/Ro 52kD117(11.7%), Anti SSB53(5.3%), Anti ScI-70 IgG27(2.7%), Anti CENP/A-B IgG25(2.5%), Anti Ku IgG30(3%), Anti PM/Scl IgG41(4.1%), ANTISPR I gG 54 29(2.9%), Anti Mi -2 IgG 3(0.3%), Anti Sp100 IgG 52(5.2%),Anti gp210 IgG 44(4.4%), Anti M2 Recombinent60(6%), Anti M2Native 46(4.6%), Anti F-actin IGG 25(2.5%), Anti Jo 1 IGG 15(1.5%), Anti Pl 7 IGG9(0.9%), Anti PL 12 IGG 9(0.9%).*

CONCLUSION:

Most common extractable nuclear antigen antibody in this study samples were found to be Anti SSA(Ro)lg G 60kD with frequency of 173(17.3%).

Keywords: *Antigen, Antibodies, Anti Nucleosome, Anti Histones*

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INTRODUCTION:

The autoimmune disorders approximately effects 3 to 5 % subjects of a population. [1] Antinuclear antibody (ANA) test and tests for specific auto antibodies to nuclear antigen play an important role in the diagnostic evaluation, prognostic assessment and monitoring of patients with autoimmune connective tissue disorder (CTD) [2]. In autoimmune connective tissue disorders the etiology and pathogenesis remains largely uncertain despite multiple linkage provided by basic sciences and clinical observations. The pathogenesis of autoimmune diseases involves a breach of immune tolerance, which may be some combinations of genetic and environmental factors that causes changes in DNA and DNA–protein structure which may influence how genes are expressed Therefore, testing for ANAs is used as a screening test in the differential diagnosis of patients when a systemic autoimmune etiology is suspected [3,4]. Timely diagnosis of Systematic autoimmune rheumatic disorder is challenging due to the wide spectrum of overlapping symptoms. Furthermore, while the frequency of ANAs is highest in patients with mixed connective tissue diseases, these antibodies are also found in patients with organ-specific autoimmune diseases (e.g., autoimmune liver diseases and Hashimoto's thyroiditis), certain infections, cancer, advanced age and in some healthy individuals [5,6,7]. Thus, ANA testing in people with a low pretest probability for a mixed connective tissue disease can cause un necessarily investigation.

Extractable nuclear antigen are over 100 different soluble nuclear and cytoplasmic antigens which can be found in saline extract of cell. [8] These antibodies are very specific for various disorders like high titers of anti–SS-A and anti–SS-B antibodies are found in SLE and ant histones may be found in drug induced SLE. [9,10] So we plan this study to see the Frequency of various extractable nuclear antigens in our local population ,it will help not only the diagnostic laboratories to purchase only those antibody profiles which are prevalent in our community and also update the knowledge of physicians about the current frequency of these autoantibodies.

MATERIAL AND METHOD:

The study was conducted in the department of chemical pathology and immunology Chughtai lab Lahore. It was a retrospective study in which data was collected from laboratory information management system Nexus pro for the duration of one year from January 2018 to December 2018.All the test were performed on the same analyzer which is based on the principle of immunoblot. A total of 1268 patients data were retrieved during one year interval.

Statistics:

All gathered data was entered and analyzed on statistical package for social sciences SPSS230. Mean ± SD was calculated for quantitative variable like age. Frequency with percentage of each individual antibody had been calculated. Chi square test was applied to see the association of gender with ten most prevalent antibodies. P value <0.05 is considered as significant.

RESULTS:

The mean age of the study population was 38.08±15.38 years. Females patients were 675(67%) which were higher than males 325(32%). Most common antibody in the study samples was Anti SSA(Ro)lgG 60kD with the frequency of 173(17.3%) (Table#1). Gender was found to be contributing for the presence of a particular type of antibody as mentioned in the Table#2.

Sr No	Antibody	Frequency
1	Anti SSA(Ro) IgG 60kD	**173(17.3%)**
2	Anti Sm/RNP IgG	124(12.4%)
3	SSA/Ro 52kD	117(11.7%
4	Anti RNP IgG 68kD/A/C	88(8.8%)
5	Anti dsDNA IgG	76(7.6%)
6	Anti Histones	72(7.2%)
7	Anti Sm IgG	63(6.3%)
8	Anti M2 Recom	60(6%)
9	Anti Nucleosome IgG	56(5.6%)
10	Anti SSB	53(5.3%)

Prevalence of twenty five antibodies were calculated which are Anti Nucleosome IgG56(5.6%), Anti dsDNA IgG76(7/6%), Anti Histones72(7.2%), Anti Sm IgG63(6.3%). Anti-Ribosome PO IgG15(1.5%), Anti PCNA IgG, 52(5.2%), Anti RNP IgG 68kD/A/C88(8.8%), Anti Sm/RNP IgG124(12.4%), **Anti SSA(Ro) IgG 60kD173(17.3%),** SSA/Ro 52kD117(11.7%), Anti SSB53(5.3%), Anti ScI-70 IgG27(2.7%), Anti CENP/A-B IgG25(2.5%), Anti Ku IgG30(3%), Anti PM/Scl IgG41(4.1%), ANTISPR I gG 54 29(2.9%), AntiMi -2 IgG 3(0.3%), Anti Sp100 IgG 52(5.2%),Anti gp210 IgG 44(4.4%), Anti M2 Recombinent60(6%), Anti M2Native 46(4.6%), Anti F-actin IGG 25(2.5%), Anti Jo 1 IGG 15(1.5%), Anti Pl 7 IGG9(0.9%), Anti PL 12 IGG 9(0.9%).

Table 2 .Association of extractable nuclear antigen with gender

Antibody	Result	Gender		P value
		Male	Female	
Anti Nucleosome IgG	Negative	271(83%)	517(76%)	**0.03**
	Borderline	42(12%)	114(16%	
	Positive	12(3.7%)	44(6.5%)	
Anti dsDNA IgG	Negative	268(82.5%)	520(77%)	**0.05**
	Borderline	41(12.6%)	95(14.1%)	
	Positive	16(4.9%)	60(8.9%)	
Anti Histones	Negative	271(83.4%)	532(78.8%)	**0.21**
	Borderline	33(10.2%)	92(13.6%)	
	Positive	21(6.5%)	51(7.6%)	
Anti Sm IgG	Negative	287(88.3%)	545(80.7%)	**0.02**
	Borderline	23(7.1%)	81(12%)	
	Positive	15(4.6%)	48(7.1%)	
Anti RNP IgG 68kD/A/C	Negative	277(85.2%)	545(80.7%)	**0.08**
	Borderline	20(6.2%)	70(10.4%)	
	Positive	28(8.6%)	60(8.9%)	
Anti Sm/RNP IgG	Negative	268(82.5%)	517(76.6%)	**0.18**
	Borderline	24(7.4%)	66(9.8%)	
	Positive	33(10.2%)	91(13.5%)	
Anti SSA(Ro) IgG 60kD	Negative	268(82.5%)	471(69.8%)	**0.00**
	Borderline	19(5.8%)	69(10.2%)	
	Positive	39(11.7%)	135(20%)	
SSA/Ro 52kD	Negative	280(86.2%)	498(73.8%)	**0.00**
	Borderline	24(7.4%)	81(12%)	
	Positive	21(6.5%)	96(14.2%)	
Anti SSB	Negative	303(92.2%)	557(82.5%)	**0.00**
	Borderline	16(4.9%)	71(10.5%)	
	Positive	6(1.8%)	47(7%)	
Anti M2 Recom	Negative	282(86%)	569(84%)	
	Borderline	20(6.2%)	69(10.2%)	0.07
	Positive	23(7.1%)	37(5.5%)	

Following antibodies has Pvalue less than 0.05 which is very significant regarding the gender distribution:Anti Nucleosome IgG, Anti SSB, SSA/Ro 52kD, Anti SSA(Ro) IgG 60kD, Anti Sm IgG.

DISCUSSION:

The rising incidence of autoimmune connective tissue disorder has occurred due to availability and usage of newer diagnostic modalities for detection of these auto antibodies. The connective tissue disorders are characterized by B cell hyperactivity (polygonal B cell activation) resulting into over production of auto antibodies against cytoplasmic, nuclear and surface antigens and immune complex formation. [11] The majority of these autoantibodies are targeted against intracellular antigens of cell nucleus (double & single stranded DNA), histones and extractable nuclear antigens (ENAs). Newer technology in clinical immunology has enabled screening for multiple autoantibodies hence making it possible (i) to diagnose the autoimmune disease in its earliest stage (ii) to intervene before serious end organ damage occurs (iii) to diagnose presence of more than one connective tissue disorder (iv) to assess the prognosis [16]. Extractable Nuclear Antigens are over 100 different soluble cytoplasmic and nuclear antigens. [8] Auto antibodies to these antigens have a partial marker function for the individual disease; hence they are associated with particular connective tissue disorders. [1,15] Certain auto antibodies have considerable disease specificity and thus can be of great diagnostic value viz. anti-ds DNA & Sm for SLE, Mi- 2 for classic Dermatomyositis, jo-1 for ant synthetase syndrome, topoisomerase- 1(Scl-70) & centromere for differentiating clinical forms of systemic sclerosis and particular organ involvement, c-ANCA(cytoplasmic antineutrophil cytoplasmic antibody) for Wegner's granulomatosis. Our study result showed that autoimmune disorders are common in female compared to male, ratio M: F = 1:2. This result is in accordance with reports from various authors viz. Malaviya et al [M: F= 1:8] and Lee et al [M: F= 1:6] [12,13]. The median age of onset in present study is 40 years while study by Malaviya et al had 24years [12]and Masi et al had 31 years [14] as a median age.

CONCLUSION AND RECOMMENDATIONS:

The incidence of autoimmune connective tissue disorder is increasing day by day due to availability of modern techniques to detect them in its earlier stage. This study is very helpful for laboratories which are dealing with connective tissue disorders diagnosis because it will help them in selection of most appropriate antibodies panel. This selection will help them in saving money as well as in diagnosing only specifically those disorders which are associated with most prevalent antibodies of our population.

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