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ESTIMATION AND TO KNOW THE ASSOCIATION OF AUTOANTIBODIES WITH DERMATOLOGICAL DISORDERS IN PATIENTS ATTENDING DERMATOLOGY OUTPATIENT DEPARTMENT

Dermatology

Dr Gaddam Sowmya Samhitha* — MBBS, Postgraduate, Department of DVL. Dr. Pinnamaneni Siddartha Institute of Medical Sciences & Research Foundation, Gannavaram, AP. *Corresponding Author

Dr Thiruveedhula Haritha — MD, Professor, Department of DVL. Dr. Pinnamaneni Siddartha Institute of Medical Sciences & Research Foundation, Gannavaram, AP.

Dr. Samanthula Haritha — MD, Professor and HOD, Department of DVL. Dr. Pinnamaneni Siddartha Institute of Medical Sciences & Research Foundation, Gannavaram, AP.

Dr. Enamurthy Sri Harsha — MD, Assistant Professor, Department of DVL. Dr. Pinnamaneni Siddartha Institute of Medical Sciences & Research Foundation, Gannavaram, AP.

ABSTRACT

Background: The presence of antinuclear antibodies (ANA) is mainly associated with autoimmune diseases and connective tissue diseases (CTD). In addition, their presence is found in healthy people. While the actual frequency of positive assays in healthy individuals varies with methodology, nevertheless, up to 20% or more of otherwise healthy people can express an ANA. The basis of this seropositivity is puzzling. One possibility is that ANA reactivity represents vagaries of the assays, allowing the detection of antibodies of either low titer or low avidity. ANA and Anti double-stranded DNA (Anti-ds-DNA) antibodies in dermatological disorders other than CTD, and Vasculitis were not studied till now to our knowledge. Hence this study has been taken up. **Aim:** Estimation and to know the association, of autoantibodies with dermatological disorders, to better understand the diseases in patients attending the dermatology outpatient department (OPD).

Objectives:

1. To find out the percentage of positivity for ANA by the Indirect Immunofluorescent Assay (IFA) method.
2. To find out the percentage of positivity for anti-ds DNA antibodies by the Enzyme-Linked Immunosorbent Assay (ELISA) method.
3. Percentage of positivity of both ANA and Anti-ds DNA antibodies in patients with dermatological problems.
4. To know the association of autoimmunity with various dermatological disorders for a better understanding of these disorders.

Materials & Methods:

A hundred patients were selected after taking written informed consent and after inclusion and exclusion criteria got fulfilled. Details of the patients were entered in the case record form and later were uploaded in a Microsoft excel sheet. One millilitre of the venous blood sample was drawn in adults of each of the age categories 18-27, 28-37, 38-47, and 48-57. The upper age limit of 57 is selected for the sake of convenience. Sera were analysed for ANA and Anti ds DNA antibodies by using IFA and ELISA methods respectively. Results were recorded and analysed. **Results:** In a total of 100 patients 11% and 13% were positive for ANA and Anti ds DNA respectively. For ANA and Anti ds DNA positivity the mean age was 34.5 ± 12.5 and 40.7 ± 10.7 respectively and females outnumbered males in both. Both ANA and Anti ds DNA were positive in 3% of which all were females with a mean age of 41 ± 9. ANA positivity was found commonly in Telogen effluvium (3), melasma (2), and Tinea (2). Anti-ds DNA positivity was found commonly in Miliaria Rubra (2), and Tinea (2). Both ANA and Anti ds DNA were positive in telogen effluvium, melasma and tinea. **Conclusion:** ANA, Anti ds DNA, both ANA and Anti ds DNA were positive in otherwise healthy dermatological patients with diagnoses like telogen effluvium, melasma, tinea, miliaria etc., to the extent of 11%, 13%, and 3% respectively. It is not known if autoantibody positivity in these diseases is just a chance association or if the autoimmune mechanism got triggered by these diseases. Prospective longitudinal studies are necessary to confirm these findings.

KEYWORDS

ANA, Anti ds DNA, dermatological disorders, autoimmunity

INTRODUCTION:

Autoantibodies to cellular constituents are the serologic hallmarks of autoimmunity and are frequently seen in systemic autoimmune diseases, including systemic lupus erythematosus (SLE), scleroderma, and polymyositis/dermatomyositis (23).

Antinuclear antibodies (ANAs) are measured by immunofluorescence staining of a cellular substrate [1]. Antinuclear antibody counts are higher in women and increase with age [2]. Sex hormones (especially oestrogens) play a significant role in the development of autoimmune diseases and predispose the female sex to more frequent occurrences of these diseases [3]. This result is also consistent with other findings in healthy control populations including a study of 500 normal individuals in Brazil showing that ANA positivity was almost twice as prevalent in females as in males [5]. The genetic factor also plays an important role. Gene products located on a not fully inactivated X chromosome can avoid presentation in the thymus and cause a break in immune tolerance [4].

ANA are present not only in patients with connective tissue disease but also in healthy people. ANA are measurable in approximately 25% of the population, and the prevalence of significantly elevated levels maybe 2.5% [6]. Most individuals with a positive ANA do not have an autoimmune disease and most also are unlikely to develop one. This is consistent with the fact that the prevalence of all autoimmune disorders is 5 to 7% [7]. Even though ANA has very high sensitivity, its specificity is quite low [8].

The standard method to determine ANA and anti-ds DNA is the indirect immunofluorescent assay method (IFA) [9, 10]. It is a technique of using the specific reactivity of antibodies with antigens to detect the circulating autoantibodies in patient serum. The specific antibodies are labelled with a compound that shows characteristic illumination when observed under an immunofluorescence microscope under UV light.

One study using the defined cut-off of >20 ELISA units (EU), found that out of the 1,159 tested, 615 (53%), individuals were ANA positive. For the subset of 401 healthy controls, the average ANA was 19.5 EU and 101 individuals were in the positive range. The percentage of the population with ANA is approximately 25% by using indirect immunofluorescence microscopy performed on Human Epithelial Cell Line (HEp-2) cells by IFA [9]. According to some reports, using IFA, ANA in low counts may appear in up to 40% of healthy people [11]. One study says that the normal population was ANA positive in 31.7% of individuals at 1:40 serum dilution, 13.3% at 1:80, 5% at 1:160 and 3.3% at 1:320 [12]. Most people with a positive ANA count are not diagnosed with autoimmune disease, and the probability of future disease is low. This is supported by the fact that autoimmune diseases occur in the general population with a frequency of 5 to 7% [7]. The

occurrence of SLE does not exceed 0.1% of the general population [13]. A positive ANA test is of much more importance if it is associated with positive anti-ds DNA.

ANA test has got high sensitivity and low specificity. To prevent the overdiagnosis of autoimmune diseases, many laboratories set the upper limit as the cut-off point for positive results [14]. There are many studies on ANA positivity in healthy individuals. But the percentage of positivity of ANA and Anti-ds DNA is not known in patients with dermatological problems. Hence this study has been taken up to fill this knowledge gap.

AIM:
Estimation and to know the association of autoantibodies with dermatological disorders in patients attending the dermatology OPD.

OBJECTIVES:
1. To find out the percentage of positivity for ANA by the IFA method.
2. To find out the percentage of positivity for anti-ds DNA antibodies by the ELISA method.
3. Percentage of positivity of both ANA and Anti-ds DNA antibodies in patients with dermatological problems
4. To know the association of autoimmunity with various dermatological disorders.

METHODS:
It is a single-centred cross-sectional study, approved by the institutional ethics committee (IEC).

Sampling Method:
Stratified Random Sampling in age groups of 18-27, 28-37, 38-47, and 48-57

Sample size: 100

Study place and Time period:
The study was conducted in our Department of Dermatology OPD from September 2022- November 2022.

Study population:
Patients attending the dermatology OPD in Dr Pinnamaneni Siddhartha Institute of Medical Sciences and Research Foundation.

Primary outcome variables :
A) Percentage of positivity of ANA in patients with dermatological problems.
B) Percentage of positivity of Anti-ds DNA antibodies in patients with dermatological problems.
C) Percentage of positivity of both ANA and Anti-ds DNA antibodies in patients with dermatological problems
D) Association between autoantibodies and various dermatological disorders will be made if autoantibodies were found to be elevated.

Secondary outcome variables:
Association between elevated autoantibodies and sociodemographic variables

Inclusion criteria:
1) Adults in the age group 18-57 years
2) Both genders
3) Patients who give written informed consent.

Exclusion criteria:
1) CTDs
2) Vasculitis

A total of 100 patients who fulfilled the inclusion and exclusion criteria and of both genders of the age groups 18-27, 28-37, 38-47, and 48-57 who attended the dermatology OPD in our institute without a prior diagnosis of any connective tissue disorders and vasculitis were selected by using a stratified random sampling method.

Details of the patient were entered in the case record form and then into an excel sheet.

ANA and ds-DNA testing:

One millilitre of the venous blood sample was drawn from each patient. Sera were analysed using an Immunofluorescence assay for ANA and the results were given based on fluorescence pattern in 1:100 dilution, if there was no reaction then it is considered as negative, trace weak fluorescence was weak positive or 1+, moderate fluorescence was positive 2+, intensified fluorescence as positive 3+, and clear fluorescence pattern as strong positive 4+. Negative denotes no specific antibodies were detected, 1+ denotes the low level of antibodies detected, 2+ denotes a moderate level of antibodies detected, 3+ denotes a significant level of antibodies detected, and 4+ denotes a high level of antibodies detected.

Sera were analysed for Anti ds DNA using ELISA. The results were given in U/ML with a positive cut-off value of 300 U/ML. The values which were above 300U/ML were taken as positive and those below were negative for Anti ds DNA. Results were analysed using statistical software.

Statistical tests:
Descriptive Statistics like percentages were used in this study. The relation between the percentage positivity of ANA and Anti ds DNA and socio-demographic variables was determined by using chi-square tests.

RESULTS:
The study includes (n=100) 44 males (44%) and 56 females (56%) with M:F = 1:1.28. The unemployed group were the maximum in the study accounting for 40%. There was a slight variation in the nativity and socioeconomic status in the study population with urban and rural at 47% and 53% respectively and rich and poor at 52% and 48% respectively. The maximum population of the study group belonged to normal (42%) followed by overweight (34%), obese (18%) and underweight (6%). Risk factors like atopy and significant sun exposure (an arbitrary value of >6 hours) were found in 43% and 57% respectively. ANA positivity was found in 11% (n=11) of patients of which 8 (72.7%) were 1+ positive and 3 (27.3%) were 2+ positive of which telogen effluvium (3) were maximum followed by facial melasma (2), tinea (2), chronic urticaria (1), alopecia areata (1), dermatosis papulosis nigra (DPN) (1) and acne vulgaris (1). Anti-ds – DNA antibodies were positive in 13 patients of which maximum patients had tinea incognito (2), miliaria rubra (2) followed by pityriasis versicolor (1), chronic urticaria with angioedema (1), telogen effluvium (1), allergic contact dermatitis (1), melasma (1), polymorphic light eruption (1), schamberg's disease (1), pityriasis rosea (1), and candida intertrigo (1). Both ANA and Anti-ds-DNA were positive in 3 patients who had telogen effluvium, melasma and tinea incognito. Of these 3 patients, ANA in tinea incognito was 2+ positive whereas in the remaining 2 it was 1+ positive.

Table 1: Age and Sex distribution:

Age in years	Male n	%	Female n	%
18-27	11	25	14	25
28-37	11	25	14	25
38-47	8	18.1	17	30.3
48-57	14	31.9	11	19.7
Total	44	100	56	100

Table 2: Classification of occupation according to Modified Kuppuswamy classification:

Occupation	Frequency	%
Unemployed	40	40
Unskilled	4	4
Semi-skilled	26	26
Skilled	15	15
Professional	15	15

Table 3: Residential status of patients :

Residence	Frequency	%
Urban	47	47
Rural	53	53
Total	100	100

Table 4: Socio-Economic Status :

Socioeconomic scale	Frequency	%
Rich	52	52
Poor	48	48
Total	100	100

Table 5: Distribution of patients as per Body Mass Index (BMI) classification by WHO:

BMI Classification	Frequency	%
18.5 kg/m2	6	6
18.5 to 24.9 kg/m2	42	42
25- 29.9 kg/m2	34	34
>30 kg/m2	18	18
Total	100	100

Table 6: Distribution of Risk Factors:

Risk factors	Frequency	%
Atopy	43	43
Sun Exposure	57	57

Table 7: Distribution of Comorbidities:

Co-Morbidities	Frequency	%
Diabetes	7	7
Hypertension	7	7
DM + HTN	6	6
Hypothyroidism	3	3
Nil	77	77
Total	100	100

Table 8: Distribution of various diseases in study:

DIAGNOSIS	FREQUENCY
INFECTIONS AND INFESTATIONS	
Tinea incognito	5
Tinea corporis	5
Tinea corporis and Tinea cruris	4
Tinea faciei	1
Extensive Tinea	1
Candidal intertrigo	2
Herpes simplex labialis	2
Pityriasis versicolor	2
Scabies	1
Subungual warts	1
Pityriasis rosea	1
Verruca vulgaris	1
DISORDERS OF APPENDAGES	
Telogen effluvium	8
Alopecia areata	4
Androgenetic alopecia	1
Acne vulgaris	4
Hidradenitis supparitiva	1
Milaria rubra	2
PAPULO SQUAMOUS DISORDERS	
Chronic plaque psoriasis	3
Psoriasis vulgaris	3
Plantar psoriasis	1
Palmoplantar psoriasis	1
Lichen planus	2
Oral LP	2
ECZEMA	
Polymorphic light eruption	4
Seborrheic dermatitis	2
Allergic contact dermatitis	2
Air borne contact dermatitis	1
Acute eczema	1
Chronic eczema	2
Nipple eczema	1
VASCULAR DISORDERS	
Chronic Urticaria	6
Acute urticaria	2
Papular urticaria	1
Schambergs disease	1
OTHERS	
Centrofacial Melasma	2
Periorbital melanosis	2
Achrocordon	2
Melasma	2
Hirsutism	1
Idiopathic pruritis	1
Prurigo simplex	1
DPN	1
Macular amyloidosis	1
Post inflammatory hyperpigmentation	1
Seborrheic dermatitis and Telogen effluvium	1
Epidermoid cyst	1
Facial melasma and Plantar psoriasis	1
Acanthosis nigricans	1
Pityriasis versicolor & Scalp Psoriasis	1

Table 9: Distribution of ANA positivity :

ANA Positivity	Frequency	%
Weak Positive	8	8
Positive	3	3
Negative	89	89
Total	100	100

Table 10: Distribution of Anti ds-DNA antibodies positivity:

ds DNA Positivity	Frequency	%
Positive	13	13
Negative	87	87
Total	100	100

Table 11: Sociodemographic profile and common diseases with positive ANA and positive Anti ds DNA:

Sociodemographic profile and common diseases		ANA Positive (n=11)	ds DNA Positive (n=13)
Age	Mean ± SD (Years)	34.5 ± 12.5	40.07 ± 10.7
Gender	M: F	1: 2.7	1: 2.2
Occupation	Unemployed	6 (54.5%)	3 (23%)
	Unskilled	1 (9%)	1 (7.6%)
	Semi-skilled	1 (9%)	3 (23%)
	Skilled	1 (9%)	6 (46.1%)
	Professional	2 (18%)	0
Residence	Urban/ Rural	1: 1.2	1: 2.2
Socioeconomic Status	Rich	6 (54.5%)	5 (38.4%)
	Poor	5 (45.5%)	3 (23%)
Risk factors	Sun Exposure	7 (63.6%)	9 (69.2%)
	Atopy	7 (63.6%)	2 (15.3%)
Co-morbidities	Diabetes	0	3 (23%)
	Hypertension	0	0
	DM + HTN	1 (9%)	0
	Hypothyroidism	2 (18%)	0
Diagnosis	1	Telogen effluvium- 3	Miliaria rubra- 2
	2	Facial melasma- 2	T. incognito- 2
	3	Tinea- 2	

Table 12: P value of Sociodemographic profile and common diseases in relation to positive ANA/Anti ds DNA/Both ANA and Anti ds DNA:

Sociodemographic profile and common diseases	ANA Positive (n=11)	ds DNA Positive (n=13)	Double Positive (n=3)	P-Value
Common diagnosis	Telogen effluvium- 3 Facial melasma- 2 Tinea- 2	Miliaria rubra- 2 T. incognito- 2	Telogen effluvium- 1 Melasma- 1 T. incognito- 1	NA
Age Mean ±SD (Yrs)	34.5 ± 12.5	40.07 ± 10.7	41 ± 9	0.25
Sex (M/F)	1: 2.7	1:2.2	0/ 3	0.85
Residence. (Urban/ Rural)	1:1.2	1:2.2	1:2	0.45
Sun Exposure	7 (63.6%)	9 (69.2%)	2 (66.7%)	0.77

DISCUSSION:

ANA are the most commonly measured biomarkers for autoimmunity and the easiest to assess at the population level. ANA tests are most commonly used to diagnose connective tissue diseases.

They also are detected in patients with organ-specific autoimmune diseases, such as autoimmune thyroiditis and hepatitis (15), certain infections and neoplasms (16), and in some individuals without diagnosed disease (16,17).

Anti-dsDNA antibodies are considered the hallmark of systemic lupus erythematosus (SLE). Antibodies to dsDNA may have a direct

pathogenic effect in lupus nephritis, lupus dermatitis and possibly also in certain aspects of cerebral lupus. How anti-dsDNA antibodies relate to the rest of the clinical components remains to be determined.

Four age groups in adults spanning from 18-57 years were selected in our study. The females outnumbered the males with a male-to-female ratio of 1:1.28 (Table 1). Most people were from rural area which may be due to the location of our institution in a rural area (Table 3). Most of the study population's body mass index was in the normal and overweight zone at 32% and 44% respectively (Table 5). The risk factors such as the history of atopy and significant sun exposure (> 6 hours) were 43% and 57% respectively (Table 6). Several dermatological disorders which come under infections and infestations, papulosquamous disorders, vascular disorders, eczemas, disorders of appendages and others like melasma, macular amyloidosis, prurigo simplex, hirsutism, DPN etc. were taken (Table 8). Of which dermatophytic infections were the maximum in number. ANA positivity in our study was 11% and these results were consistent with the study done by Davidson et al [7]. In this study, the mean age in patients with ANA positivity in this study was 34.5 ± 12.5 and females outnumbered males with a ratio of 2.7:1 which were consistent with other studies [18,19,20,21,22]. Most of the patients with ANA positivity were unemployed 54.5% followed by professionals (18%).

The risk factors viz., atopy and significant sun exposure were present independently in 70% of population with ANA positivity. Both significant sun exposure and atopy together were positive in 27.27% in patients with ANA positivity.

Out of 11 (n=11) patients with ANA positivity, two (18%) had hypothyroidism out of which one patient was 1+ positive and the other was 2+ positive. One patient with ANA positivity had both Diabetes Mellitus and Hypertension positive. So, comorbidities were found in three (27.23%) in total 11 (100%) with ANA positivity.

The most common diagnoses associated with ANA positivity (n=11) are telogen effluvium (3, 27.23%), Facial melasma (2, 18.2%), and tinea (2, 18.2%).

Anti-ds-DNA positivity in our study was 13% from total 100 study population. In this study, the mean age in patients with Anti ds DNA positivity was 40.07 ± 10.7 and females outnumbered males with a ratio of 2.2:1. Most of the patients with Anti ds DNA positivity were skilled (46.1%) followed by semi skilled (23%) and unemployed (23%). The risk factors atopy and significant sun exposure were present in 15.4% and 69.2% respectively in patients with Anti ds DNA positivity. Out of 13 patients three (23%) had comorbidities and all the three were diabetics. The most common diagnoses associated with Anti ds DNA positivity were miliaria rubra (2, 15.4%) and tinea incognito (2, 15.4%). There is no significant association between ANA and Anti ds DNA positivity and sociodemographic profile ($p>0.05$).

There are studies on ANA positivity in normal healthy people but there are no studies on ANA positivity in patients with dermatological disorders. There no studies on Anti ds DNA positivity in normal healthy people or patients with dermatological disorders. In our study we have found that there is 11% of ANA positivity and 13% of anti-ds DNA positivity in patients with various dermatological disorders and 3% had both the ANA and Anti ds DNA were positive.

All the patients with positive autoantibodies found in this study had dermatological issues like tinea, miliaria etc. None of the above had any systemic complaints pertaining to CTD. Hence it is not known if these people would develop any CTDs or Vasculitis in future. This autoantibody positivity makes it imminent to follow-up these patients for a long time to know about the natural course of antibodies.

Dermatological diseases like telogen effluvium, tinea, melasma, miliaria etc were associated with autoantibody positivity in this study. It is not known if autoantibody positivity is just a chance association or if there is activation of autoimmune mechanism by these diseases. Further workup of above diseases to look for positive autoantibodies is necessary to come to any conclusion.

Limitations:

1. Cross sectional design of this study does not allow us to know about long-term follow-up. Prospective longitudinal long-term studies are essential to know about phenotypical presentation of autoantibody positivity.
2. ELISA method of Anti ds DNA antibody estimation has lower sensitivity and specificity than compared to that of Immunofluorescent Assay methods.
3. ANA positivity was estimated in IFA method at 1:100 dilution in this study. Different dilutions were not estimated.
4. Small sample size of 100.

CONCLUSION:

ANA positivity (by IFA method) was found in 11% of patients attending dermatology OPD with telogen effluvium, melasma and tinea commonly. Anti-ds DNA positivity (by ELISA method) was found in 13% of patients attending dermatology OPD with miliaria rubra, tinea, and pityriasis versicolor commonly. Both ANA and Anti ds DNA positivity were found in 3% of dermatology outpatients with telogen effluvium, melasma and tinea incognito.

It is not known if above mentioned dermatological diseases trigger the autoimmune mechanism and lead to phenotypic presentation of CTDs in future or if it is a chance association. Long-term prospective longitudinal studies are essential to know about the autoimmune triggering mechanism in dermatological diseases like telogen effluvium, tinea, melasma, miliaria etc.

Conflicts Of Interest: NONE.

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