The high Percentages of Anti-thyroid Antibodies Positive SLE Patients at Sheikh Zayed Hospital, Lahore (Pakistan)

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ABSTRACT

BACKGROUND & OBJECTIVES: Systemic lupus erythmatosus (SLE) is an autoimmune disease and more than 100 auto antibodies have been detected in SLE. The present study was designed to determine the presence of anti-thyroid antibodies (ATA) in SLE patients. **METHODS:** It was a descriptive study and 42-SLE patients positive for anti-nuclear antibodies (ANA) and or anti-ds DNA (ds-DNA) antibodies were selected from Department of Rheumatology, Sheikh Zayed Hospital Lahore. Blood sample was collected and ATA was determined by indirect immunofluorescence technique. **RESULTS:** Thirty-nine (39) (92.9%) and 32 (76.2%) patients had ANA and ds-DNA antibodies respectively. On comparison, it was not statistically significant. Twenty-three (23) (54.76%) subjects had ATA and on comparison, it was not statistically significant. All the positive patients for ATA were females. **CONCLUSION:** Sensitivity of ANA and ds-DNA positive suspected SLE subjects to have ATA in their serum was 74%. About 55% of SLE patients were positive for ATA.

Key words: Systemic lupus erythematosus, anti-thyroid Antibodies, Immunofluorescence

الملخص

المقدمة والأهداف: تعتبر الذئبة الحمراء أحد أمراض المناعة الذاتية، وهنالك أكثر من ١٠٠ من الأجسام المضادة الذاتية تم اكتشافها في الذئبة الحمراء. هذه الدراسة قد تم تصميمها لتحديد وجود اجسام مضادة للغدة الدرقية في مرضي الذئبة الحمراء. **منهج الدراسة:** دراسة وصفية تم فيها دراسة ٤٢ مريض ايجابي للأضداد النووية (ANA) و / أو مضادات الدي ان اي (DS-DNA) تم اختيارهم من قسم لأمراض الروماتيزمية بمستشفى الشيخ زايد بلاهور. تم فحص وجود اجسام مضادة للغدة الدرقية في الدم بواسطة تقنية الومضان المناعي الغير مباشر. **النتائج:** ٣٩ مريض (٩٢,٩٪) كان ايجابي للأضداد النووية (ANA)، و٣٢ مريض (٧٦,٢٪) كان ايجابي لمضادات الدي ان اي (DS-DNA). وعلى سبيل المقارنة، لم يكن الفرق يعتد به إحصائيا. ٢٣ مريض (٥٤,٧٦٪) كان ايجابيون لوجود اجسام مضادة للغدة الدرقية (ANA) وعلى سبيل المقارنة، لم يكن ذات دلالة إحصائية. جميع المرضى الإيجابيون لوجود اجسام مضادة للغدة الدرقية (ANA) كانوا إناث. **الخلاصة:** حساسية وجود اجسام مضادة للغدة الدرقية (ATA) لدي المرضي الايجابيين للأضداد النووية (ANA) و لمضادات الدي ان اي (DS-DNA) ٧٤٪ . و حوالي ٥٥٪ من مرضى الذئبة الحمراء كانوا ايجابيون النتائج للأضداد النووية (ANA)

INTRODUCTION

Autoimmune diseases occur when physiological tolerance of self-antigens is lost and thereafter, human body cannot discriminate between self and non-self-tissue. Multiple factors such as environment, heredity, viruses, drugs, etc. may contribute towards an autoimmune disease.[1] Autoimmune diseases are categorized into systemic and organ specific diseases and production of auto-antibodies is the hallmark of these diseases[2] where auto-antibodies results in variety of pathologic manifestations.[3]

Systemic lupus erthematosus (SLE) is a systemic multi-organ autoimmune disorder, where microvascular inflammation is one of the characteristic features.[4] Like other autoimmune disorders, exact cause of SLE is not known, but multiple factors such as racial, genetic, hormonal, and environmental are associated with it.[5, 6]

The American College of Rheumatology, in 1982, established criteria to diagnose SLE and in 1997 they revised it. In 2012 the Systemic Lupus International Collaborating Clinics (SLICC) group revised and validated the ACR SLE classification criteria of 1997. They classified a person as having SLE in the presence of biopsy-proven lupus nephritis with ANA or anti-dsDNA antibodies or if 4 of the diagnostic criteria, including at least one clinical and one immunologic criterion, have been satisfied.[7]

In SLE, auto antibodies contribute towards pathogenesis of disease manifestations. Systemic lupus erythematosus (SLE) patients show defective early B cell tolerance checkpoints and therefore

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accumulate self-reactive and polyreactive antibodies.[8] Antinuclear antibodies (ANA) are found in nearly all the active SLE patients but antibodies to native ds-DNA have been suggested as the specific marker for diagnosis of SLE.[9]

The annual incidence of SLE averages 5 cases per 100,000 population. The Centers for Disease Control and Prevention (CDC) estimates a range between 1.8 and 7.6 per 100,000 persons per year in the continental United States.[10] The reported prevalence ranges from 52 cases per 100,000 population.[11] It is consistent with the 2005 CDC estimates of 161,000 definite cases and 322,000 probable or definite SLE cases in the United States to estimates as high as 1:1000.[10]

The Lupus Foundation of American estimates prevalence to be up to 1.5 million cases, which likely reflects inclusion of milder forms of this disease. According to a 2008 report from the National Arthritis Data Working Group, approximately 250,000 Americans have definite SLE.[12]

In SLE, there is high level of interferon secretion, that results in an aberrant expression of major histocompatibility complex antigen by thyrocytes which provokes an autoimmune response and production of antithyroid antibodies.[13]

In SLE, more than 100 auto antibodies have been detected.[14] Antibodies against thyroid antigens have been documented in a number of non-organ-specific rheumatological disorders such as SLE.[15] Therefore, in SLE anti-thyroid activity by auto antibodies may result in thyroid disorders.[16] Autoimmune thyroid disorders are characterized by anti-thyroglobulin (ATA) and anti-thyroid peroxidase autoantibodies.[17] These two antibodies are collectively named as anti-thyroid antibodies (ATA).[18]

In SLE, prevalence of autoimmune thyroid disorders was documented between 3.9%–24% and the frequency of anti-thyroid antibodies was between 11-51%.[19] Another study suggested that 14% anti-thyroid antibodies are present in SLE.[20]

Therefore a study was designed to determine the percentage of anti-thyroid antibodies positive SLE patients visiting Sheikh Zayed Hospital Lahore Pakistan. For the detection of anti-thyroid antibodies different laboratory techniques have been used, e.g. ELISA and indirect Immunofluerescence (IIF). IIF method is more reliable and specific compared to ELISA[21], therefore IIF technique was opted for this study.

Materials and Methods

Samples:

Blood samples of 42 SLE patients from the Department of Immunology & Rheumatology Sheikh Zayed Hospital, Lahore were collected. These patients were diagnosed according to the criteria of American College of Rheumatology. These SLE patients were positive for anti-nuclear (ANA) antibody or anti double stranded DNA antibody (anti-ds DNA).

Determination of ATA:

The anti-thyroid antibodies were determined in the Department of Immunology, University of Health Sciences (UHS) Lahore by the indirect immunofluorescence technique by using slides coated with thyroid tissue (Orgentec Company Limited Germany).

The antibodies of the diluted patient samples and controls were allowed to react specifically with the antigens of the tissue sections immobilized on the slides. After an incubation period of 30 minutes at room temperature(RT) (15-30°C), unbound serum components were removed by a wash step. The bound antibodies were allowed to react specifically with anti-human IgG conjugated to Fluorescein-isothiocyanat (FITC). After an incubation period of 30 minutes at RT (15-30°C) excessive conjugate was separated from the solid-phase immune complexes a wash step. Stained slides were read using a fluorescence microscope(Olympus CX41). The study was approved by the Ethical Review Committee and Advanced Study and Research Board of UHS and Sheikh Zayed Hospital Lahore. Written informed consent of each participant was obtained before collecting their blood sample.

Data Analysis: The data was entered and analysed using Predictive Analytics Software (PASW) 18.0. Mean±SD for quantitative variables, while frequencies, percentages and graphs were given for qualitative variables. A p-value of <0.05 was considered as statistically significant. OpenEpi, Version 3 was used to calculate sensitivity of different parameters.

RESULTS

Among 42 diagnosed SLE patients, there were 41 (98%) females and only 1 (2%) male. The mean ± SD of age of SLE patients was 27±7.1 years (range 15–45). Thirty nine (93%) SLE patients were positive for ANA while 03(7%) were negative for these antibodies. Among the studied population, 32 (76%) SLE patients were positive for anti-ds DNA antibody while 10(24%) patients were negative for this antibody. Anti-thyroid antibodies were present in 23(54.76%) patients, while 19(45%) patients were negative for this antibody. Number, percentage and comparison of ATA positive and ATA negative with ANA and anti-dsDNA is presented in Table 1. The presenting complaints of the studied population are presented in Table 2.

DISCUSSION

The current study included 42 SLE patients that comprised of 41 females (98%) and 1 (2%) male patient and they were between 15-45 years with the mean age of 27 years. Regarding the percentage of gender and mean age of the subjects, current study is in agreement with the study of Konho et al., (1989)[22] who also included 98% SLE females and their mean age was 27.4 years. Inclusion of more females in the current study could entail the higher percentage of ATA in this study as the prevalence and incidence of thyroid disorders is influenced primarily by gender.[23]

In the current study among 42 SLE patients, 39 (93%) patients were positive for ANA. These results are in agreement with the findings of Gibson et al.[24] because they also reported presence of ANA in 95% of SLE patients. The current study is also in agreement with the study of Gill et al[25] because they documented that more than 99% of patients with systemic lupus erythematosus have an elevated ANA. However, the current study is not in agreement with the study of Tektonidou et al[26] who reported ANA in 35% of SLE patients. The probable reason for this discrepancy could be due to the larger number of patients included in their study (168) as compared to the current study which included 42 patients.

In the current study 23 (55%) of SLE patients were positive for ATA. The current study is in agreement with the study of El-saadanya et al[27] who reported that Anti-TPO abs and anti-TG abs were more frequently detected in SLE 85% and 55% respectively. Magaro et al[28] also reported antithyroid antibodies in 45% of their SLE patients. The small difference between the percentages of ATA in both the studies could be due to the methodology used for the detection of these antibodies because in the current study IIF technique was used that is more specific as compared to ELISA technique which was used in the other studies. The study of Pyne et al[20] is not in agreement with the current study as they documented anti-thyroid antibodies in 14% of SLE subjects.

In the current study, among 42 SLE patients, 19 (49%) patients had proteinuria which is in agreement with the study of Akbarian et al[29] because they documented presence of proteinuria in up to 55% of SLE patients. However, current study is not in agreement with the study of Christopher et al[30] bezcause they documented presence of proteinuria in 77% of SLE patients. The difference in the percentages of the subjects with proteinuria in two studies could be due to the differences in the stages of SLE patients included in these studies. In the current study, 28 SLE patients (67%) had complaints of arthritis that is in agreement with Cervera et al[31] as they documented arthritis in 71% of SLE patients.

In the current study, 15 SLE patients (36%) had malar rash and 9 (21.4%) patients had oral ulcer. These findings are in agreement with the study of Boddaert et al., (2004)[32] who documented malar rash as

Table 1: Number, percentage and composition of ATA positive and ATA negative with ANA and anti-dsDNA

		ATA Positive	ATA Negative	Total	p value
ANA	Positive	21 (91.3%)	18 (94.7%)	39 (92.9%)	0.667
	Negative	2 (8.7%)	1 (5.3%)	3 (7.1%)	
Anti-ds DNA	Positive	18 (73.7%)	14 (73.7%)	32 (76.2%)	0.729
	Negative	5 (21.7%	5 (26.3%)	10 (23.8%)	

Table 2: Number, percentage and comparison of presenting complaints of ATA posivite and negative subject

ATA Positive	Presenting complaint	Age Range				p value
		15-30		31-45		
		Positive n(%)	Negative n(%)	Positive n(%)	Negative n(%)	
	Oral Ulcer	3 (18.8)	13 (81.2)	3 (42.9)	4 (57.1)	0.318
	Arthritis	7 (43.8)	9 (56.2)	5 (71.4)	2 (28.6)	0.371
	Malar Rash	7 (43.8)	9 (56.2)	4 (57.1)	3 (42.9)	0.667
	Proteinuria	8 (50)	8 (50)	4 (60)	3 (40)	0.999
	ANA	14 (87.5)	2 (12.5)	7 (100)	0 (0.0)	0.557
	Anti-ds DNA	12 (75)	4 (25)	6 (85.7)	1 (14.3)	0.550
ATA Negative	Oral Ulcer	2 (14.3)	12 (85.7)	0 (0)	2 (10.5)	0.999
	Arthritis	11 (78.6)	3 (21.4)	5 (100)	0 (0)	0.530
	Malar Rash	3 (21.4)	11 (78.6)	1 (20)	40(80)	0.999
	Proteinuria	4 (28.6)	10 (71.4)	3 (60)	2 (40)	0.301
	ANA	14 (100)	0 (0.0)	4 (80)	1 (20)	0.263
	Anti-ds DNA	10 (71.4)	4 (28.6)	4 (80)	1 (20)	0.999

31% in their studied population. However, current study is not in agreement with Cervera et al[31] who documented malar rash as 79% in SLE patients.

Conclusion

Anti-thyroid antibodies were detected in high percentage (55%) of SLE patients.

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Recommendation

Level of anti-thyroid and other antibodies should be determined in larger group of different autoimmune disorders.

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