

Diagnostic accuracy of antinuclear antibodies and anti-double stranded DNA antibodies in patients of systemic lupus erythematosus presenting with dermatological features

Attiya Tareen*, Nighat Naqi**, Aamir Afzal†, Uzma Malik*

* Department of Dermatology, Fauji Foundation Hospital, Rawalpindi

** Department of Pathology, Fauji Foundation Hospital, Rawalpindi

† Foundation University Medical College, Rawalpindi

Abstract *Objective* To determine the diagnostic accuracy of antinuclear antibodies (ANA) and anti-double stranded DNA (dsDNA) antibodies in systemic lupus erythematosus patients presenting with dermatological features.

Methods In this cross-sectional study, 82 patients diagnosed as having systemic lupus erythematosus (SLE) per American College of Rheumatology (revised criteria 1997) from October 2012 to October 2013 and fulfilling the study's inclusion criteria were enrolled. These patients were first assessed by the dermatologist for four dermatological features, namely malar rash, discoid rash, photosensitivity and oral ulcers and were then referred to Department of Pathology for assessment of serological markers i.e. ANA and anti-dsDNA antibodies. The diagnostic accuracies of serological markers were analyzed to determine which one of the two serological markers is associated with any of the four dermatological features under observation in our study.

Results Out of 82 SLE patients, 77 (93.9%) patients were females and 5 (6.1 %) were males. Male to female ratio in this study was 1:15. Mean age of patients was 34.91 years and ranged between 8-62 years. Photosensitivity, malar rash, oral ulcers and discoid rash were found in 97.5%, 85.3%, 76.8% and 46.3% patients, respectively. ANA was not found to be statistically significantly associated with any of the dermatological features under observation in our study. However, anti-dsDNA antibodies were strongly associated with photosensitivity 0.024 ($p<0.05$) and malar rash was 0.003 ($p<0.05$).

Conclusion The study proves that anti-dsDNA antibodies have high diagnostic accuracy for photosensitivity and malar rash in SLE patients and can be used alone with confidence in patients presenting with these two dermatological features, without burdening the lab with a lengthy serological evaluation.

Key Words

Systemic lupus erythematosus (SLE), dermatological features, serological markers.

Introduction

Systemic lupus erythematosus (SLE) is a

multisystem autoimmune disorder and therefore has diverse clinical manifestations of variable intensity.¹ Importance of dermatological changes in SLE cannot be overstressed as according to 1997 American College of Rheumatology (revised) criteria used for classification of SLE, four out of eleven are related to skin manifestations.² Dermatological features are

Address for correspondence

Dr. Attiya Tareen
Department of Dermatology,
Fauji Foundation Hospital, Rawalpindi
E mail:attiyatareen@yahoo.com

very common and are known to be seen in 72% to 85% of SLE patients.³ Autoantibodies testing is used to confirm the diagnosis of SLE and they are also part of diagnostic criteria of SLE. Antinuclear antibodies (ANA) and anti-double stranded DNA (dsDNA) antibodies are important antibodies determinants, in the evaluation of patients suspected of having SLE.⁴ These serological variables were selected for this study because of their proved diagnostic significance in SLE. No study has yet been conducted to determine diagnostic accuracy of ANA and anti-dsDNA antibodies in SLE patients presenting with dermatological features and sufficient data are lacking in this context in Pakistani population. This study was, therefore, conducted with the objective to determine diagnostic accuracy of the two serological markers in Pakistani SLE patients presenting with four dermatological features namely photosensitivity, malar rash, discoid rash, and oral ulcers all included in diagnostic criteria of SLE.

Methods

The study was conducted at Department of Dermatology in collaboration with Department of Pathology (Immunology), Fauji Foundation Hospital, Rawalpindi from October 2012 to October 2013. A total of 82 patients diagnosed as having SLE as per American College of Rheumatology revised criteria 1997 and fulfilling the inclusion criteria were included in the study. SLE patients who had none of the four dermatological features were excluded from the study. These patients after being assessed by the dermatologist for four dermatological features, namely, malar rash, discoid rash, photosensitivity and oral ulcers were then referred to Department of Pathology (Immunology) for assessment of serological markers i.e. ANA and anti-dsDNA antibodies.

For laboratory testing venous blood sample was collected and serum separated for quantitative detection of ANA and anti-dsDNA antibodies. ANA was tested by ANA screen ELISA (IMMCO Diagnostics Inc., U.S.A) and anti-dsDNA antibodies were tested by dsDNA Antibody ELISA Immulisa Enhanced Kit by (IMMCO Diagnostics Inc., U.S.A). The cut off value for ANA for negative, borderline and positive cases were <20 IU/ml, 20-25 IU/ml and >25 IU/ml, respectively. The cut off value for anti dsDNA antibodies for negative, borderline and positive cases were <50 IU/ml, 50-60 IU/ml and 60 IU/ml, respectively. Statistical Package for Social Sciences (SPSS) version 17 was used for statistical analysis of the data. A *p* value of <0.05 was considered statistically significant.

Results

82 SLE patients were included in this study. Mean age of patients was 34.91 years and ranged between 8-62 years. Out of 82, SLE patients, 77 (93.9%) patients were females and 5 (6.1 %) were males. Male to female ratio in this study was 1:15.

Photosensitivity, malar rash, oral ulcers and discoid rash were found positive in 97.5%, 85.3%, 76.8 % and 46.3 % patients, respectively as shown in **Figure 1**.

Association of ANA and anti-dsDNA antibodies with dermatological changes in systemic lupus erythematosus patients are shown in **Table 1**.

The sensitivity, specificity, positive and negative predictive value of ANA for photosensitivity, malar rash, oral ulcers and discoid rash is given in **Table 2**. The sensitivity, specificity, positive and negative predictive value of anti-dsDNA antibodies for photosensitivity, malar rash, oral ulcers and discoid rash is given in **Table 3**.

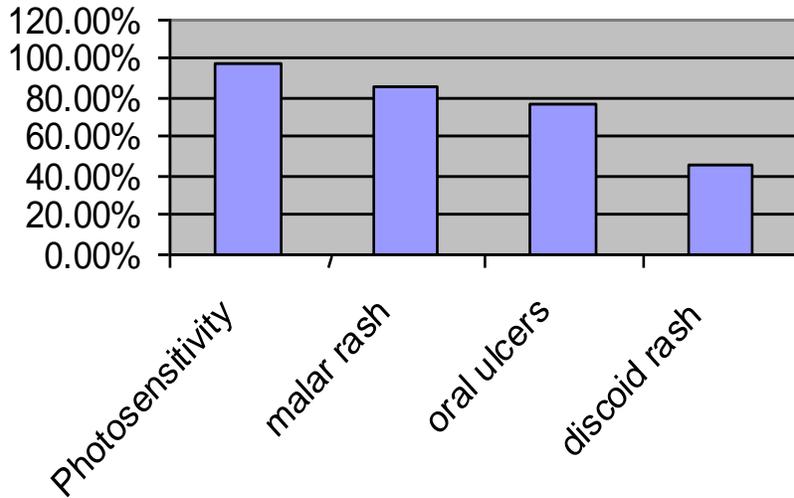


Figure 1 Frequencies of dermatological features in SLE patients.

Table 1 Association of ANA and anti-dsDNA antibodies with dermatological features in SLE patients.

Serological markers	Photosensitivity	Malar rash	Oral ulcers	Discoid rash
ANA positive patients	32 (39.2%)	26 (31.7%)	24 (29.2%)	16 (19.5%)
ANA negative patients	8 (9.7%)	4 (4.8%)	5 (6.09%)	3 (3.6%)
Anti-dsDNA positive patients	27 (32%)	18 (21.9%)	23 (28.0%)	12 (14.6%)
Anti-dsDNA negative patients	13 (15.8%)	12 (14.6%)	6 (7.3%)	7 (8.5%)

Table 2 Association of ANA with Dermatological features in SLE.

Parameter	Photosensitivity	Malar rash	Oral ulcers	Discoid rash
Sensitivity	80%	86.6%	82.7%	84.2%
Specificity	21.9%	30.2%	26%	27%
Positive predictive value	49.2%	40%	36.9%	24.6%
Negative predictive value	52.9%	76.4%	70.5%	82.3%
p value	0.873	0.209	0.564	0.544

Table 3 Association of anti-dsDNA antibodies with dermatological features in SLE.

Parameter	Photosensitivity	Malar rash	Oral ulcers	Discoid rash
Sensitivity	67.5%	60%	79.3%	63.1%
Specificity	12.5%	10%	17.1%	18.6%
Positive predictive value	42.1%	28.1%	35.9%	18.7%
Negative predictive value	27.7%	33.3%	66.6%	61.1%
p value	0.024	0.003	0.838	0.074

Discussion

The mean age of patients in our study was 34.9 years and ranged between 8-62 years. Our results are very near to the results of the study conducted earlier on Pakistani SLE patients showing mean age of 30.35 years.⁵ Another international study has shown that the disease is frequently seen between 20 and 40 years, with a female predominance of 3:1.⁶ A study on Indian

population has reported that 98% patients were female and 84% patients were under the age of 40 years.⁷ The female to male ratio in this study was in line with the one conducted earlier showing high female preponderance with a ratio ranging from 7:1 to 15:1.⁸

Out of the four dermatological features under study, photosensitivity (97.5%) was the most common dermatological manifestation in our

SLE patients followed by malar rash (85.3%), oral ulcers (76.8%) and discoid rash (46.3%), respectively. However, the study conducted by Agrawal *et al.*⁷ has shown malar rash to be the most common clinical feature where 71.3% patients presented with malar rash followed by photosensitivity (63.2%) and oral ulcers (42.5%). A study done by Malaviya *et al.*⁹ analyzing 1366 SLE patients from different part of India reported significantly high proportion of SLE patients presenting with mucocutaneous manifestations specially malar rash (58.5%). According to Ahmed *et al.*¹⁰ fever was the most common presenting feature seen in 100% SLE patients, followed by arthritis (98%), malar rash (64%) and oral ulcers (58%). This difference in dermatological presentation of SLE patients in various studies could be due to different geographical and ethnic groups.

ANA was positive in 79.3% and anti-dsDNA antibodies in 78% of SLE patients showing statistically insignificant difference in sensitivity of both serological parameters for SLE patients presenting with dermatological features. Also the ANA was negative in 20.7% and anti-dsDNA was negative in 22 % cases at the time of diagnosis of SLE patients showing statistically insignificant difference between the specificities of the two markers having skin changes associated with SLE. These results state that both ANA and anti-dsDNA antibodies or either one can be used to identify SLE patients presenting with skin manifestations. Likewise Hussain's study on Pakistani SLE patients has also concluded that no single clinical or immunological feature can be used to confirm diagnosis.⁵ Another study states that ANA and anti-dsDNA antibodies can be positive for years before clinical symptoms appear thus proving our point that both serological markers have equal diagnostic accuracy for SLE.¹¹ A previous

study shows different results than ours, reporting sensitivity of ANA for SLE patients to be 93% to 95% and specificity 57%.¹² Similarly, another study reported sensitivity of anti-dsDNA antibodies for SLE patients to be 57.3% and specificity of anti-dsDNA antibodies 97.4% much higher than ours.¹³ The possible explanation for this difference in sensitivities and specificities of the two serological markers in our study when compared with previous ones, could be due to the difference in technique used for determination of both serological markers. In our study both serological markers were analyzed by ELISA technique, which is less specific for antibodies detection as compared to gold standard immunofluorescence testing.¹⁴ Other studies have reported that while ELISA assays have advantage of being less labor intensive and usage of detecting a panel of common specific autoantibodies, they are not 100% sensitive when compared with immunofluorescence.^{15,16} Thus the technique used in our study can attribute to the low sensitivity and specificity of both serological markers as compared to other studies .

When the diagnostic accuracy of ANA was determined for the four dermatological features independently none was found to be statistically significantly ($p>0.05$) associated with ANA. However, on analyzing the diagnostic accuracy of anti-dsDNA antibodies testing for the four dermatological features in SLE patients it was found that anti-dsDNA antibodies were statistically significantly ($p<0.05$) associated with photosensitivity and malar rash. The p value of anti-dsDNA antibodies for photosensitivity was 0.024 ($p<0.05$) and p value of anti-dsDNA antibodies for malar rash was 0.003 ($p<0.05$). Thus our study has proved that anti-dsDNA antibodies testing has high diagnostic accuracy for photosensitivity and

malar rash in SLE patients and can be used with confidence in patients presenting with these two dermatological features.

The positive predictive value of ANA was highest for photosensitivity and negative predictive value was highest for discoid rash in our study group. Similarly in our study the positive predictive value of anti-dsDNA antibodies was highest for photosensitivity and negative predictive value was highest for oral ulcers. Nevertheless we cannot ignore a previous study that states that ELISA technique produces more false positive results, which may reduce the positive predictive value of the test.¹⁷

Conclusion

This study would prove helpful in the accurate and early diagnosis of SLE patients presenting with dermatological features with anti-dsDNA antibodies.

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