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Immunological Studies on Systemic Lupus Erythematosus

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Abstract: Abstract:The main work of this study was the Immunological Studies on Systemic Lupus Erythematosus collected from different patients admitted to Mansoura hospitals. Then, this study was to define the disease through stereotyped diagnosis by detecting antinuclear antibodies (Anti-ds-DNA, ANA) by ELISA of samples collected from patients diagnosed with systemic lupus erythematosus in Immunology and Rheumatology Clinics of University Hospital Mansoura in Egypt. Systemic lupus erythematosus is an autoimmune disease in which the immune system attacks various organs and tissues of the body such as the kidneys, heart, lungs, brain, blood and skin. 50 samples (66.7%) were positive for ANA, while there were 25 samples (33.3%) were negative for ANA, while all samples were 100% positive for Anti-ds-DNA using ELISA.

keywords: Systemic lupus erythematosus,ANA, Anti ds- DND

1.Introduction

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterized by deviant action of the protected system **[1**] and presents with a wide range of clinical manifestations, including renal, dermatological, neuropsychiatric, and cardiovascular symptoms **[2**]. The overall estimated prevalenceof adult SLE patients in Egypt was 6.1/100,000 populace (1.2/ 100,000 males and 11.3/100,000 females). **[3].**Unfortunately, there appears to be a trend of increasing SLE prevalence with time **[4**]. Healthcare-related costs of SLE are linked to disease cruelty and the types of organ(s) involved **[5].**The diagnosis of SLE is based on characteristic clinical findings of the skin, joints, kidneys, and the central nervous system, as well as on serological strictures such as antinuclear antibodies (ANA), in precise antibodies to ds-DNA. The numerous medical signs do not constantly occur instantaneously and may advance at any point of the disease. In the early points, surgeons from several castigations often proposition several disparity diagnoses, or categorize only one part of the ailment without identifying the indications as part of SLE. Illness, lethargy, and arthralgia are the most frequently occurring nonspecific symptoms at sickness onset; additional joint distension or a "butterfly rash"—mostly in women of childbearing age—should rapid contemplation of SLE **[6].**

2. Materials and methods

Collection of samples

The study was performed on 75 patients. Blood samples were collected from patients who were admitted to different Mansoura University Hospitals.

Detection of Anti- Nuclear Antibody (ANA) Screen

1- Strips were placed into the holder.

2- Test examples were diluted by adding 10µl of the sample to 200 µl of taster diluents mix well.

3- 100 µl of watery sera were dispensed. Calibrator and panels into the appropriate wells. For the mixture blanks were bestow 100 µl sample diluents in 1A well position. Was Rap the box to remove air fizzes from the fluid and mix fine, reared for 20 minutes at room temperature.

4- Fluid from all bores were uninvolved and wash wells three times with 300-350 µl of 1x wash buffer and blotted on absorbance paper or daily towel.

5- 100µl of enzyme conjugate were allotted to each well and gestate for 20 minutes at room temperature.

6- Enzyme conjugate from all wells were disinterested and washed wells three times with 300-350 µl of 1x shampoo buffer and blotted on absorbance paper or paper towel.

7- 100µl of TMB substrate were dispended and incubated for 10 minutes at room temperature and 100 µl of stop resolution was added.

Detection of Anti ds-DNA:

1- A diluted samples and pre diluted calibrator and controls were applied to wells.

a- Controls were applying 100 µl of pre diluted was calibrated and controls to assigned wells and add100µl of sample diluents as a blank control.

b- Patient samples were applying 100 µl of diluted patient serum (1: 100 in sample diluents) to assigned wells.

2- Wells was Incubated and shacked plate gently then incubated for 30 minutes at room temperature (20 – 25 ^{o}c).

3- Samples were incubated and discard after 30 minutes of incubated, was by inverting plate and rapidly flicking the liquid away from the plate.

4- Wells were washed rinse and flick the wells 3times with ~ 350 µl of wash solution and discard remove all liquid before proceeding.

5- Added 100 µl of conjugate reagent was added to all wells. Excess transferred conjugate reagent after use was discarded.

6- Incubated wells were shacked gently then incubated for 30 minutes at room temperature, (20 – 25 ^{o}c).

7- After 30 minute incubation were conjugate reagents by inverting plate and rapidly flicking the liquid away from the plate.

8- Wells were washed 3 times with ~ 350

9- µl of wash solution and discard remove all liquid before proceeding.

10- Color in add 100 µl of TMB substrate to each well was developed and discard excess transferred TMB after use.

11- Incubated wells were shacked gently then incubated for 30 minutes at room temperature, (20 – 25 ^{o}c).

12- After 15 minute color development, and adding 100 µl of stop solution to each well to stop the color development.

13- Reading results were read within 15 minutes with an EIA reader set to 450 nm**.**

3. Results and Discussion

Collection of samples from patients.

In this study was carried out, on seventy-five systemic lupus erythematosus were from patients admitted to different Mansoura University Hospitals and seventy-five controls healthy.

Results recorded in **Table (1)** and **Figure (1)** shows that samples 50 (66.7%) were the samples giving positive for ANA while there samples 25 (33.3%) were negative for ANA.

Table (1): Results of ANA for SLE patients**.**

ANA in SLE patients (total n.=75)			
Positive		**Negative**	
No.	%	No.	%
50	66.7	25	33.3

Normal value of ANA (0.9 -1.1µl)

Results recorded in **Table (2)** and **Figure (2)** shows that samples 75 (100%) were the samples giving positive for Anti ds-DNA while no sample were negative for Anti ds-DNA.

Table (2): Results of Anti ds-DNA for SLEpatients

Anti dsDNA in SLE patients (total n.=75)			
Positive		**Negative**	
No.	%	No.	%
75	100	zero	0

Normal value of Anti ds-DNA (Less than 0.9 µl)

Table (3) illustrates that there is statistically significant difference between cases and control group as regard ANA and anti ds-DNA expression with 66.7%of the studied cases are positive ANA expression and 100% of cases are positive anti ds-DNA.

Table (3): Comparison of ANA between cases and control group

	Control No=75	Cases No=75	Test of significance
ANA-ve+ve	75(100%)0	25(33.3%) 50(66.7%)	x^2=75p<0.001*
Anti dsDNA-ve+ve	75(100%)0	075(100%)	x^2=150p<0.001*

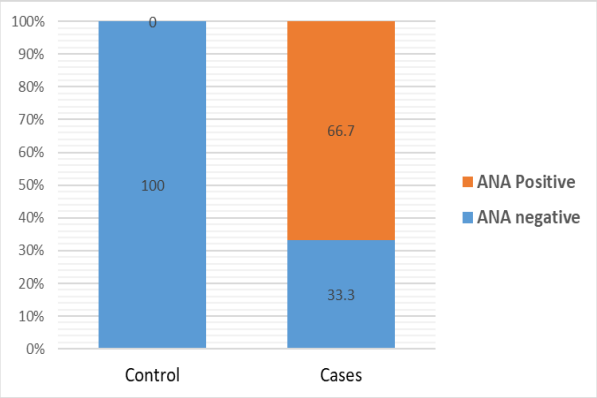

Fig (1): ANA distribution among studied groups.

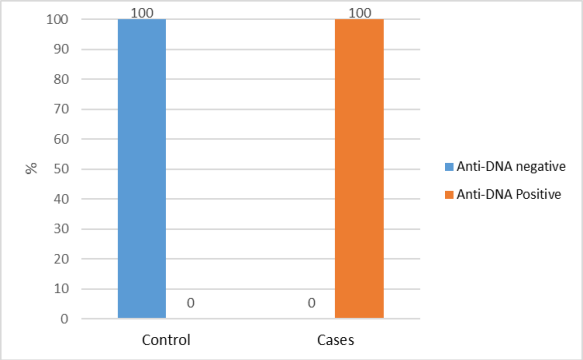

Fig (2): Anti ds-DNA distribution among studied groups.

Discussion

Systemic lupus erythematosus (SLE) or lupus is a chronicautoimmune disease, characterized by a spectrumof immunological abnormalities and production ofautoantibodies resulting in widespread inflammationcausing tissue and organ damage. The prevalence ofSLE is 6.5 to 178.0 per 100,000 globally with varying epidemiologic information **[7].**

In this study, seventy-five systemic lupus erythematosus samples isolates were isolated from patients admitted to different Mansoura University Hospitals. Among these samples,50 (66.7%) were the samples giving positive for ANA while there samples 25 (33.3%) were negative for ANA. On analyzing the demographic data of ourpatients, several features were found to be similar when compared to their peers from the Middle East and across the globe, including age of onset **[7].**and female predominance **[8].**

Results in this training displayed that there is no statistically significant difference between cases with positive ANA expression and cases with negative expression as regard their age

The frequency of helpful ANA and anti-ds-DNA was like to several previous studies. *Serological* showing for auto antibodies exposed that around 66.7% of SLE patients were sero positive for ANA lower related to that informed in other Arab studies and anti ds-DNA antibodies were sero positive 100% for patients. Such findings confirm the diagnosis of SLE in the contemporary womanly patients. Former lessons also confirmed that the common of SLE patients mature ANA, and amid them are anti ds-DNA antibodies, which are of precise notice in SLE outstanding to their great latent in finding of ailment **[9].** Serological showing for auto antibodies discovered that round 80% of SLE patients were sero positive for ANA and anti ds-DNA antibodies. Such discoveries check the finding of SLE in the present female patients. Former studies also proved that the mutual of SLE patients matures ANA, and among them are anti ds-DNA antibodies, which are of exact curiosity in SLE due to their high likely in diagnosis of bug **[10].**.

Conclusion

The results obtained here concluded that hematological, renal, and musculoskeletal were the most common clinical manifestations, whereas anti-dsDNA antibody was the most frequent autoantibody of the SLE patients addition, our study suggests that SLE patients having a combination of high titers of anti ds-DNA, anti-Nuc antibodies could be more prone to develop multiple clinical manifestations contributing to the disease complexity of the patients.

50 (66.7%) of the samples were giving positive for ANA while there samples 25 (33.3%) were negative for ANA. Thereforesamples 75 (100%) were the samples giving positive for Anti ds-DNA while no sample were negative for Anti ds-DNA.

4. References

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