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## RESEARCH ARTICLE

### Detection of anti-ds DNA Antibodies in Iraqi Patients Suspected with Systemic Lupus Erythematosus (SLE).

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#### Abstract

**Introduction:** Systemic lupus erythematosus (SLE) is autoimmune disease where autoantibodies are frequently targeted against intracellular antigens of the cell nucleus double and single stranded DNA (ds-DNA and ss-DNA, respectively), histones, and extractable nuclear antigens (ENAs). The detection of anti ds-DNA antibodies are highly specific for SLE and are therefore very important in the diagnosis and clinical monitoring of the disease and evaluation its activity in patients. The main aim of this study is estimation the role of anti double stranded DNA (anti-ds DNA) in the diagnosis and prevalence of Systemic Lupus Erythematosus (SLE) disease in Iraqi patients. **Materials & Methods:** A total of 154 Iraqi patients with SLE symptoms were attended to Al-Yarmouk hospital in Baghdad from March to June 2015. Venous blood sample was collected and serum separated for quantitative detection for anti-dsDNA antibodies using Enzyme-Linked Immunosorbent Assays (ELISA) commercial Kit by (Generic Assay gmbh/Germany). The Statistical Analysis System- SAS (2012) was used to effect of different factors in Anti dsDNA antibody.

**Results:** A total of 154 blood samples suspected SLE, there was a significant differences between male 31(20.1%) and female 123(79.9%). In addition the age group less than 30 years (Mean  $\pm$  SE 36.30  $\pm$  10.82) was differ statistically from the age group more than 50 years (13.05  $\pm$  1.34). A full data of Anti dsDNA antibody was obtained for 154 patients, 19 (12.34%) was positive and 135 (87.66%) was negative without any positive result in control group. The prevalence incident of positive samples higher in female 16(13.01%) more than 3 (9.68%) in male versus negative results (86.99%, 90.32%) respectively. The positive Anti dsDNA antibody was highest in 30-50 years group 13 (16.05%) compared to other age groups (6, 11.32% and 0.00%) respectively.

**Conclusion:** Results study reveal the differences between negative and positive Anti dsDNA antibody and all factors including gender and age groups in Iraqi patients. Current study useful in the early diagnosis of SLE patients presenting anti-dsDNA antibodies using ELISA technique.

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#### Introduction:-

Systemic Lupus Erythematosus (SLE) is a chronic autoimmune disease which affects multiple organ systems, resulting in tissue damage by auto-antibodies (antibodies that attack healthy self proteins (1-3). Systemic lupus erythematosus (SLE) is autoimmune disease where autoantibodies are frequently targeted against intracellular antigens of the cell nucleus double and single stranded DNA (ds DNA and ss DNA, respectively), histones, and extractable nuclear antigens (ENAs) (4).

Many different parts of the body can be affected, SLE most often affects the skin, joints and kidney. The clinical manifestations of lupus are diverse but common symptoms consist fatigue, joint pain & swelling, fever, skin rash

(butterfly rash) on cheeks , sensitivity to light , head ach , hair loss, anemia and blood clotting problems (5,6). Recent 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(7).

Anti-double stranded DNA antibody is one of a group of autoantibodies called antinuclear antibodies (ANA). Normally, antibodies protect against infection, but autoantibodies are produced when a person's immune system fails to adequately distinguish between "self" and "non-self."(8).

Autoantibodies are usually polyclonal—of mixed isotype, affinity, and avidity—and are often directed against multiple targets. Different assays detect particular antibody properties, which are often quite different, and the clinical importance of this for pathogenesis or diagnosis is rarely fully understood. All these assays require careful validation to determine whether they perform adequately for detecting human autoantibodies , SLE diagnosis in lab is a perfect example of this problem(4).

Auto antibodies against double stranded DNA are a hall mark of systemic lupus erythematosus (9). The detection of anti ds-DNA antibodies are highly specific for SLE and are therefore very important in the diagnosis and clinical monitoring of the disease and evaluation it's activity in patients (10,11). Increasing antibody titers are a ssociated with disease exacebration, especially with the risk of glumerulonephritis, immune complexes consist of ds-DNA & IgG anti ds-DNA antibodies seems to play a pivotal role in the pathogenesis of lupus nephritis (11).

The main aim of this study is to investigate the presence of anti-dsDNA antibodies in serum with individual clinical manifestations using Enzyme-Linked Immunosorbant Assays (ELISA). In addition to estimation the role of anti double stranded DNA (anti-ds DNA) in the diagnosis and prevalence of Systemic Lupus Erythematosus (SLE) disease in Iraqi patients.

## Materials & Methods:-

### Patients:-

A total of 154 Iraqi patients with SLE symptoms were attended to Al-yarmouk hospital in Baghdad from March to June 2015 who were included in the present study . Among them 123 were females and 31 were males, in addition to 20 healthy blood donors were considered as control.

### Samples collection:-

For laboratory testing venous blood sample was collected and serum separated for quantitative detection for anti-dsDNA antibodies using Enzyme-Linked Immunosorbant Assays (ELISA) commercial Kit by (Generic Assay gmbh/Germany).The cut off value of anti-dsDNA antibodies were assessed according to the manufacturer's instructions as positive, negative and borderline cases ( >35 IU/ml, < 30 IU/ml and 30-35 IU/ml), respectively (Table 1).

**Table 1:-** Reference values of anti-dsdna antibodies using Enzyme-Linked Immunosorbant Assays

Anti-ds DNA	Values (IU/ml)
Positive	>35
Negative	<30
Grey zone	30-35

### Statistical Analysis:-

The Statistical Analysis System- SAS (2012) was used to effect of different factors in Anti- dsDNA antibody. Least significant difference (LSD) test was used to significant compare between means, Chi-square test was used to significant between percentages in this study (12).

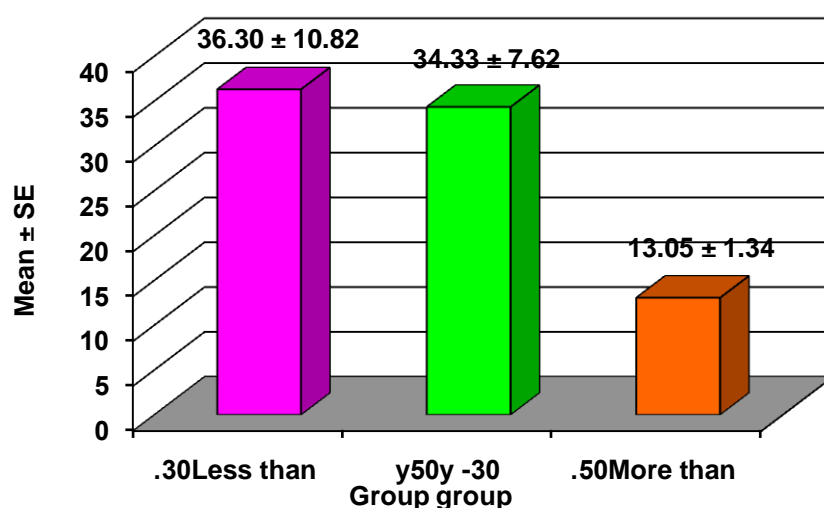
### Results:-

Out of 154 blood samples suspected SLE age was ranged between (4-75) years. According to the study group , there was a significant differences between male 31(20.1%) and female 123(79.9%) with P-value =0.0209(Table 2).In addition the age group less than 30 years (Mean  $\pm$  SE 36.30  $\pm$  10.82) was differ statistically from the age group More than 50 years (13.05  $\pm$  1.34 ) with LSD value 18.758 \* ,P-value =0.0519 (Figure 1).

**Table 2:-** Number and percentage of gender in this study

Sex	No (%)	Mean $\pm$ SE of Anti ds DNA
Male	31(20.1)	16.96 $\pm$ 3.80
Female	123(79.9)	36.10 $\pm$ 6.76
LSD value	---	16.201 *
P-value	---	0.0209

\* (P&lt;0.05).

**Figure 1: Age groups of patients suspected SLE**

A full data of Anti - dsDNA antibody was obtained for 154 patients, 19 (12.34%) was positive for SLE, 135 (87.66%) was negative and without any positive result in control group. The prevalence incident of positive samples higher in female 16(13.01%) more than in male 3 (9.68%) versus negative results (86.99%, 90.32%) respectively.

According to the age groups the positive Anti- dsDNA antibody was highest in 30-50 years group 13 (16.05%) compared to other age groups (6,11.32% and 0.00%) respectively. Finally, results study reveal that high significant differences between negative and positive Anti- dsDNA antibody in all factors including samples groups, gender and age groups (Table 3).

**Table 3:-** Distribution of samples study according to the result of Anti -ds DNA antibody.

Factors	No.	+ve (%)	-ve (%)	Chi-square
Samples Groups	Patients	154	19 (12.34%)	13.96 **
	Control	20	0 (0.00%)	15.00 **
Gender	Male	31	3 (9.68%)	14.63 **
	Female	123	16 (13.01%)	13.85 **
Age	Less than 30	53	6 (11.32%)	14.02 **
	30-50	81	13 (16.05%)	13.40 **
	More than 50	20	0 (0.00%)	15.00 **

\*\* (P&lt;0.01).

### Discussion:-

The anti-ds DNA antibody population is regarded as a marker for SLE, rheumatology criteria for the disease include antibody binding to DNA as a classification & an overestimation of biologic & diagnostic impact of these antibodies (13). Because they were considered useful in diagnosing SLE, anti DNA antibodies became part of the college of rheumatology classification criteria. In addition to serving as a laboratory marker for SLE, anti DNA antibodies may directly contribute to pathogenic processes such as lupus glomerulonephritis (14).

The circulating DNA that form immune complexes with antibodies in SLE patients displays a characteristic fragmentation pattern of apoptosis by gel electrophoresis. These findings have suggested an interplay of apoptosis & circulating DNA in the pathogenesis of SLE (15, 16).

In addition, There is evidence that IgG & ds DNA antibodies are pathogenic especially for renal tissue damage, because of their high efficiency for complement fixation, high affinity for antigen, charge & cross reactivity cause SLE disease activity (17,18)

Table (3) shown that anti-ds DNA test was negative in all control group which represents the healthy people. The results of this study in line with study in Asia, they reported that the results of the infection with SLE were 3.2-19.3% in India, Japan and Saudi Arabia (19). In Iraq this disease accounted for 0.67% of the medical admissions and it was consider the third most frequent inflammatory rheumatoid (1).

SLE affects more women than men & also women experience worsening of symptoms during pregnancy & with their menstrual period. Both of these observations have led some medical professionals to believe in causing SLE (5).

Some study disagree with present study, one of them in Norway, results shown that the percentage of infection with SLE was 5.8% in all cases with high percentage (91.0%) in female compare to 0.7% in male (20).

While women comprise the majority of lupus patients across all age groups, the differences in rates of diagnosis by sex drops for prepubescent & post-menopausal age groups, the fact that rates of lupus diagnosis spike for women during their reproductive years suggests a possible of correlation between certain sex hormones & SLE. Women with lupus have abnormally high levels of estrogen & low levels of progesterone (21). In addition, some men with SLE may have lower levels of testosterone & high levels of estrogen than men who do not have lupus disease (22).

However biological sex differ in immune function, especially those induced by sex hormones, are less likely explanation of sex differences. Recent studies suggested chromosomal basis & environmental effects exposure for both sex differences in the incidence of lupus (23).

Most autoantibodies increase during active disease, but few prospective data are currently available to justify treatment on the basis of rising titres. Further randomized prospective studies are required to examine the importance of antibody isotype and affinity in the monitoring of SLE by individual assay methods. The most important aspect of the appropriate use of laboratory assays is to become familiar with the limitations of the technology currently in use in your local laboratory, and to consult with your clinical immunologist in cases of doubt, preferably before commencing serological screening.

Results in this study hint that the differences between the incident with lupus autoimmune disease in Iraqi patients and other nations contribute to the genetic predisposing and environmental conditions which is differ from country to another.

### **Conclusion:-**

Recent study reveals differences between negative and positive Anti- dsDNA antibody with all factors including gender and age groups in Iraqi patients. Current study useful in the early diagnosis of SLE patients presenting anti-dsDNA antibodies using ELISA technique.

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