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THE RELATIONSHIP OF ENA PROFILE AND SOME NEW IMMUNOLOGICAL MARKERS IN LUPUS NEPHRITIS PATIENTS IN KERBALA PROVINCE

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Abstract

Introduction: *Lupus nephritis is an immune complex Glomerular Nephritis (GN) that develops as a frequent complication of SLE, the pathogenesis of lupus nephritis involves a variety of pathogenic mechanisms. The external etiology of systemic lupus is based on multiple combinations of genetic variants that compromise those mechanisms normally assuring immune tolerance to nuclear auto antigens, the increased incidence of ESRD underlines the importance of early diagnosis in this difficult to control disease with unpredictable course*
This study aimed to distinguished between the patients with primary glomerulonephritis from the secondary glomerulonephritis (SLE) by using the Phospholipase A2 receptor test (PLA2R), and aimed to use the ENA screen test to detect The patients with naïve SLE from the patient with SLE associated with other autoimmune disease, also measurement the the concentration of NGAL, VCAM, ANA, ds-DNA by using ELISA method, this research included eighty-five female patients.

Materials and Methods: *A cross section study of patients with Systemic lupus erythematosus was conducted. Imam AL Hasan Al Mujtaba Hospital in Karbala city during period from November 2022 to March 2023.*

Results: *The Positive PLA2R was observed in **5** (5.9%) which is present as the primary glomerulonephritis while Negative PLA2R present in **80** (94.1%) present as secondary glomerulonephritis (Systemic lupus Erythromatous) as shown in Table (1).*
*The results also show that **77%** of patients who were anti-ENA negative (Naïve SLE) while **23%** of anti-ENA positive patients which conceder (SLE associated with other autoimmune disease) as shown in Figure (1).*
*The results of current study have illustrated a significant increase of NGAL concentration in Sever Lupus nephritis group, compared with Moderate Lupus nephritis and other organ SLE group, the mean of NGAL was (**1027.53**± 259.01 ng/ml) (**768.82**± 228.8) (**715.89**± 173.9 ng/ml**)** respectively as shown in Table (2).*

*The results of current study have illustrated un significant difference of VCAM in Sever Lupus nephritis compared with Moderate Lupus nephritis and other organ SLE group, the mean of VCAM was (**21.43**± 5.83 ng/ml) (**13.6**± 2.63) (**13.56**± 12.28 ng/ml**)** respectively as shown in Table (2).*

*The concentration of Anti- Nuclear Antibodies (ANA) is higher in sever lupus nephritis in compeer with moderate Lupus Nephritis and other organ SLE the mean of ANA (**6.22**± 2.41U/ml) (**2.11**± 0.77 U/ml) (**1.03**± 0.19 U/ml**)** respectively Table (2).*
*The concentration of Anti-double strand deoxyribonucleic acid (anti-dsDNA) is higher in sever lupus nephritis in compeer with moderate Lupus Nephritis and other organ SLE group the mean of ds-DNA (**425.47**± 180.33 U/ml) (**233.8**± 93.73 U/ml) (**77.13**± 30.91U/ml**)** respectively Table (2).*

Conclusion: *The positive PLA2R was conceder primary membranes nephropathy that exclude from result while the negative result conceder SLE lupus nephritis that detect their results. Also increase the concentration of vascular cell adhesion molecule VCAM-1, NGAL gradually with increase of disease severity so we suggest a significant correlation with lupus nephritis activity also both ANA and ds-DNA as the result showed that increase so we can suggest that there is a relationship between there criteria and the flare up of disease.*

Keywords: *PLA2R: Phospholipase A2Receptor, ENA: Extractible Nuclear Antigen, ANA: Anti-Nuclear Antibody, anti-ds DNA: Anti-double strand deoxyribonucleic acid, VCAM: vascular cell adhesion molecule, NGAL: Neutrophil gelatinase associated Lipocaline, GN: Glomerular Nephritis, ESRD: End Stage Renal Disease.*

INTRODUCTION:

Lupus nephritis is an immune complex Glomerular Nephritis (GN) that develops as a frequent complication of SLE (Bao *et al.,*2011). The morbidity and mortality of LN is considerable, with up to (10 %) of the patients developing end-stage renal disease (ESRD, defined as dialysis or transplantation) (Hanly *et al.,* 2016). Late diagnosis of lupus nephritis is correlated with a higher frequency of renal insufficiency (Esdaile *et al*., 1994) The increased incidence of ESRD underlines the importance of early diagnosis in this difficult to control disease with unpredictable course (Faurschou. *et al*., 2006). The pathogenesis of lupus nephritis involves a variety of pathogenic mechanisms. The external etiology of systemic lupus is based on multiple combinations of genetic variants that compromise those mechanisms normally assuring immune tolerance to nuclear auto antigens (Bao *et al.,*2011). Lupus Nephritis is one of consequences of SLE, which is an autoimmune disease characterized by overproduction of antibodies to self-antigens, which are mostly derived from cell components like the nucleus, cytoplasm ribosomes, and cell membranes, the extra renal etiology of systemic lupus is based on multiple combinations of genetic variants that compromise those mechanisms normally assuring immune tolerance to nuclear auto antigens. This loss of tolerance becomes clinically detectable by the presence of antinuclear antibodies (Yu *et al.,*2010).

The intra renal etiology of lupus nephritis involves antibody binding to multiple intra renal auto antigens rather than the deposition of circulating immune complexes. Tertiary lymphoid tissue formation and local antibody production add to intra renal complement activation as renal immunopathology progresses (Cairns *et al.,* 2003). The immune Complex Mediating renal Immuno-pathology and nonspecific activation of autoreactiveB cells explains the polyclonal autoantibody response leading to the LN, the full house pattern of IgA ,IgM and IgG deposits (Schwartzman&Putterman ;2012).Immune complex deposits in the glomeruli are primarily responsible for the inflammatory process and lead to glomerular damage, If deposited in subendothelial space and the mesangium, immune complexes will activate the complement system(cause hypocomplementemia) and generate chemotaxis or attractants (C5a and C3a), The result is an influx of neutrophils and mononuclear cells that secrete proteases, reactive oxygen species, and proinflammatory cytokines and chemokines, causing glomerular injury(Fiehn *et al.,* 2003).

MATERIALS AND METHODS

Study design and setting

This study cross –sectional of patients with Systemic lupus erythematosus was conducted.in Imam AL Hasan Al Mujtaba Hospital in Karbala city during period from November 2022 to March 2023. eighty female patients (85) participants were enrolled in this study including three groups involved in this cross -sectional study according to clinical diagnosis, patients were Sever Lupus Nephritis was taken [35] Female patient, the second group Moderate Lupus Nephritis include [20] female patient and the third group was Other organ SLE [30] Female patient.

Ethical consideration

The research followed the guidelines set forth by the Department of Clinical Laboratories at the University of Karbala's College of Applied Medical Sciences for dealing with biological substances and dangerous microorganisms. After acquiring the necessary authorization from the hospital administration and patients, the samples for this investigation were taken from patients at the Imam AL Hasan Al Mujtaba Hospital in Karbala city**.**

Statistical analysis:

The quantitative data are expressed as mean ± standard deviation. The Student t-test was used to compare these data between discharged well patients and those required ICU admission. Binomial data were presented as frequency percentages and analyzed by Chi square test. Receiver operating characteristic (ROC) curve was used to evaluate the predictive value for all markers that had a significant variation between the two groups at admission in predicting ICU admission. All data were analyzed with SPSS for windows, v.25.0; IBM Corp, Armonk, New York, USA.

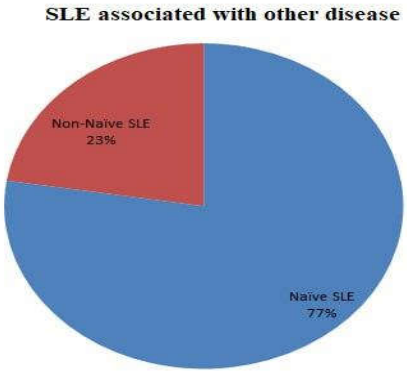
RESULTS

Table (1) S LE patients with Lupus Nephritis and other organs depend on Phospholipase A2 receptor test in Female patients

	Sever LN	Moderate LN	Other organ SLE	Total Number
Positive	5	0	0	5
Negative	30	20	30	80
Statistical analysis	X^2= 7.5; Df=2; P=0.022			

The Positive PLA2R was observed in **5** (5.9%) which is present as the primary glomerulonephritis while Negative PLA2R present in **80** (94.1%) present as secondary glomerulonephritis (Systemic lupus erythromatous) as shown in Table (1).

Figure (1) the naïve SLE and the SLE associated with other autoimmune disease



The results also show that **77%** of patients who were anti-ENA negative (Naïve SLE) while **23%** of anti-ENA positive patients which conceder (SLE associated with other autoimmune disease) as shown in Figure (1)

Table (2) Concentration of ANA and anti-dsDNA in patients with Lupus Nephritis and other organs

Markers	**Sever LN**	**Moderate LN**	**Other organ SLE**	Normal range *
NGAL Mean ±SD	**1027.53**± 259.01	**768.82**± 228.8	**715.89**± 173.9	(283-990) ng/ml
VCAM Mean ±SD	**21.43**± 5.83	**13.6**± 2.63	**13.56**± 12.28	(5.1-19.2) ng/ml
ANA Mean ±SD	**6.22**± 2.41	**2.11**± 0.77	**1.03**± 0.19	<1.0Neg >1.0Pos U/ml
ds-DNA Mean ±SD	**425.47**± 180.33	**233.8**± 93.73	**77.13**± 30.91	<30 neg >50 pos U/ml

The results of current study have illustrated a significant increase of NGAL concentration in Sever Lupus nephritis group, compeer with Moderate Lupus nephritis and other organ SLE group, the mean of NGAL was (**1027.53**± 259.01 ng/ml) (**768.82**± 228.8) (**715.89**± 173.9 ng/ml**)** respectively ,and un significant increase in VCAM for Sever Lupus nephritis compare with Moderate Lupus nephritis and other organ SLE group, the mean of VCAM was (**21.43**± 5.83 ng/ml) (**13.6**± 2.63) (**13.56**± 12.28 ng/ml**)** respectively as shown in Table (2).these increase is associated with increase the concentration of Anti- Nuclear Antibodies (ANA) is higher in sever lupus nephritis in compeer with moderate Lupus Nephritis and other organ SLE the mean of ANA (**6.22**± 2.41U/ml) (**2.11**± 0.77 U/ml) (**1.03**± 0.19 U/ml**)** respectively, and the concentration of Anti-double strand deoxyribonucleic acid (anti-dsDNA) is higher in sever lupus nephritis in compeer with moderate Lupus Nephritis and other organ SLE group the mean of ds-DNA (**425.47**± 180.33 U/ml) (**233.8**± 93.73 U/ml) (**77.13**± 30.91U/ml**)** respectively Table (2).

DISCUSSION:

The current study agreement with Svobodova; (2013) that show detection of PLA_2R antibody in serum has an almost 100% specificity for the diagnosis of PLA_2R1-associated membranous nephropathy (Svobodova*; et al* 2013)

This study conformity to the study of Beck ;(2009) that show serum PLA2R autoantibody was found only in Idiopathic memberance nephropathy but not in other renal diseases (Beck, *et al* 2009).

It has been reported about the value of either serum PLA2R antibody or glomerular PLA2R antigen to diagnosis of primary MN with 50–80% sensitive and almost 100% specific (Dai H; et al 2015).

Phospholipase A2 receptor is overexpressed in renal tissue epithelial cells of Primary membranes nephropathy patients, the expression of anti-PLA2R antibodies also follows, and a series of studies has confirmed that the levels of anti-PLA2R antibodies in PMN patients are significantly higher than those in normal and non-MN patients (Ramachandran *et al*.,2021).

The anti-PLA2R antibody has many biological characteristics and can cause complement system activation, podocyte injury, and basement membrane damage when combined with PLA2R on the glomerular podocyte membrane leading to the emergence of large amounts of proteinuria (Mcquarrie EP, 2015).

This result agreement with Jeong that show the ENA can be used to diagnosed autoimmune disease (Jeong *et al.,* 2018).This study conformity to the study of (Khater 2022) that show ENA autoantibodies were crucial and need to be correlated with clinical diagnosis and other serological testing for early diagnosis and intervention of the autoimmune disease (Khater & Al Sheik ,2022)Certain autoimmune diseases are characteristically related to the existence of anti-ENA antibodies, these autoantibody associations can help to distinguish between different autoimmune diseases and aid in the diagnosis of autoimmune disorders (Orton *et al.,* 2004).

The result exhibited that VCAM-1 is expressed on endothelial cells and tubules and it participates in the migration and recruitment of leucocytes (Seron *et al.,* 1991).
Vascular Cell Adhesion Molecule - 1 is expressed on endothelial cells and tubules and it participates in the migration and recruitment of leucocytes, it could distinguish active LN from inactive disease in adults and is sensitive to change in status (Stanley *et al.,* 2020).
The current study agreement with study of Singh;(2012) that found higher VCAM- 1 in those patients with class V disease at entry (Singh *et al.,* 2012)
The study of Parodis;(2020) found high U-sVCAM-1 levels appear to reflect SLE disease activity, U-sVCAM-1 showed ability to distinguish SLE patients with active renal involvement from patients with quiescent or no prior nephritis, High U-sVCAM-1 levels may indicate patients at increased risk for long-term renal function loss. (Parodis *et al.,* 2020).
The result of current study agreement with Nakhjavani; (2019) that found that the serum NGAL was significantly higher in SLE individuals, Furthermore, NGAL was even more elevated in SLE patients with LN when compared to those without nephritis Additionally, individual sera biomarker was related to histologic findings in LN, especially those representing LN activity (Nakhjavani *et al.,* 2019).
The current study conformity to the study of Parikh;(2011) that show the NGAL has been the most widely studied biomarker in acute renal injury and has been demonstrated to possess an excellent diagnostic performance, previous studies have shown that concentrations in urine and serum of NGAL represent sensitive, specific, and highly predictive biomarkers for acute renal injury after cardiac surgery (Parikh *et al.,* 2011).
As far as other renal disorders are concerned, the current study is in accordance with Xiang and Bolignano;(2012) who documented increased level of serum NGAL in patients with chronic kidney disease (CKD) e.g. in polycystic kidney disease, IgA nephropathy, dysplasia, obstruction, Lupus Nephritis and glomerulonephritis (Xing and Hogquist, 2012).
This study demonstrated that high titers of ANA are most often associated with active SLE (Kavanaugh, 2000) said that this test is one of the most common tests used by physicians to diagnosis SLE. The anti-nuclear antibody (ANA) is heterogeneous group of antibodies produced against variety of antigens within the cell nucleus.A positive ANA test does not automatically mean lupus but it shows that immune system is making an antibody that reacts with components of body's cells, ANA positive is not mandatory for the diagnosis, because most people with SLE have ANA, but most patients with ANA do not have SLE, and may not relate to the patient's symptoms but were indicated to other autoantibodies that may present in patient serum. (Hyoun *et al.,* 2009). This finding was similar to many studies (Maher, 2013).
These results show that increase in anti dsDNA antibody concentration prior to disease exacerbations of SLE is part of a restricted immune response or merely the consequence of polyclonal B cell activation (Ter Borg *et al*., 1991). Moreover, Giasuddin *et al*., (1991) have mentioned that the anti dsDNA antibodies are present in 85.3% of SLE patients.
For many years, the anti-dsDNA antibody assay has been regarded as the serological gold standard in the diagnosis and assessment of disease activity in patient with SLE (Isenberg, 2004). The prevalence of anti-dsDNA in this study was 75%. This is in consistent with previous data which reported that anti-dsDNA reactivity was between 40 -80% of SLE patients (Ravirajan *et al.,* 2001).

CONCLUSION:

The positive PLA2R was conceder primary membranes nephropathy that exclude from result while the negative result conceder SLE lupus nephritis that detect their results. Increase the concentration of vascular cell adhesion molecule (VCAM-1) gradually with increase of disease severity so we suggest a significant correlation with lupus nephritis activity Neutrophil gelatinase-associated Lipocaline (NGAL) can be used as an early diagnostic marker of acute kidney injury.Also increase the concentration of both ANA and ds-DNA as the result showed that increase so we can suggest that there is a relationship between there criteria and the flare up of disease, the result of ENA screen confirm that 77% that naïve SLE so the renal failure disease from the lupus while the 23% of patients that associated with other autoimmune disease may be this autoimmune disease reasons for renal failure.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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