Utility of Urinary Ferritin as a Marker for Systemic Lupus Erythematosus

Usama Ragab [1], Yasser Abd El-Monem El-Hendy [1],Ghada El-Sayed Amr[2],Esraa Sameh Ibrahim Awadallah[3] Ahmed Abdulsaboor[2], Ahmed Noaman[1]

1 Internal Medicine Department, Faculty of medicine, Zagazig University, Egypt.

2 Clinical Pathology Department, Faculty of medicine, Zagazig University, Egypt.

3Nephrology Department, Zagazig General Hospital, Egypt.

Abstract

Background: Systemic lupus erythematosus (SLE) is a chronic autoimmune inflammatory disease with a wide spectrum of clinical and serological manifestations caused by autoantibody production, complement activation, and immune complex deposition. Urinary biomarkers are easily obtained and probably are best at reflecting the current renal status, as they specifically represent local inflammatory activity.The aim of this study was to evaluate the value of urinary ferritin/creatinine ratio (UFCR) in diagnosis of Systemic lupus erythematosus. **Methods:**This study was conducted in the internal medicine department of Zagazig university hospitals on 36 patients complaining of SLE diagnosed according to the American College of Rheumatology (ACR) 1997 revised criteria for the classification of SLE, and they were compared with 18 healthy control participants (16 of them were females and two were males) with mean age 32.28±6.03.**Results:**Urinary ferritin creatinine ratio (UFCR)was significantly higher in the SLE group than the control group. There was a statistical significance +ve correlation between UFCR and SLEDAI score, serum ferritin and serum creatinine among SLE group. **Conclusion:**UFCR level can be considered as a potential biomarker for SLE.

Key words: Urinary Ferritin Creatinine Ratio -Biomarker -Systemic lupus erythematosus.

Corresponding Author Name: ESRAA SAMEH IBRAHIM AWADALLAH

Phone Number: 01117788260Email:dr-esraasameh@yahoo.com

Introduction:

SLE is a chronic autoimmune inflammatory disease characterised by a wide range of clinical and serological manifestations caused by autoantibody production, complement activation, and immune complex deposition. SLE is a rare disease with a prevalence of 20–200 per 100,000 person-years and an incidence of 1–10 per 100,000 person-years. It primarily affects young and middle-aged women[1].

Urinary biomarkers are simple to obtain and are likely the best indicator of current overall health since they directly reflect local inflammatory activity[2].

Ferritin, a ubiquitously expressed iron storage protein first found in 1937 by Vilém Laufberger [3], is well known for its ability to acquire and store up to 4500 atoms of iron [4]. Ferritin is a soluble protein with a molecular weight of 450 kDa that is not routinely filtered by the glomerulus. It is made up of 24 subunits[5].

Ferritin levels are raised in autoimmune diseases, infections, cancer, liver disease, and cardiovascular and cerebrovascular diseases, in addition to iron excess[6].

Ferritin has gotten a lot of attention in autoimmune illness research in recent years. Antiphospholipid syndrome (APS), rheumatoid arthritis (RA), and adult-onset Still's disease all have elevated serum ferritin levels as an acute-phase reactant. Several research have found

higher serum ferritin levels in SLE patients, but few have looked into the clinical implications of urine ferritin in SLE patients[5].

The purpose of this study was to see how useful the urine ferritin/creatinine ratio (UFCR) is in diagnosing systemic lupus erythematosus.

Methods

Study design

Retrospective case control study that wascarried out in Internal Medicine Department, Faculty of Medicine,Zagazig University Hospitals between February2021 and August2021.

Study Population

The participants in this study ranged in age from 18 to 45 years old and included both females and males. Thirty-six patients were diagnosed with SLE and met at least four of the 11 American College of Rheumatology (ACR) SLE criteria. We separated them into three groups, with group I consist of 18 SLE patients who did not have LN (17 were females and 1 was male). They were admitted owing to lupus flares with arthritis, proteinuria of less than 0.5 g/day, no hematuria, and no urinary casts sediment, and they all have normal kidney function and estimated GFR of more than 90 mL/min/1.73m2. There were 18 SLE patients with LN in Group II (18 were females with no males),proteinuria > 0.5 g/day was present in all patients, and some have increased serum creatinine levels (> 1.1 mg/dl in females and > 1.2 mg/dl in males). Group III consisted of 18 apparently healthy control individuals who were age and sex matched to the patients' groups.

Inclusion Criteria:Male and female patients between the ages of 18 and 40. The American College of Rheumatology (ACR) 1997 criteria for the diagnosis of SLE are met by all lupus patients. Proteinuria of more than 0.5 g/24 h in patients with lupus nephritis. People who were in good health and didn't have any clinical or laboratory signs of a chronic medical condition as healthy control group.

Exclusion Criteria: In nearly six months, none of the participants had taken any iron supplements. Patients with diabetes, cardiovascular and cerebrovascular illness, liver disease, blood diseases, and other serious co-morbidities. Patients with overlap syndrome, urinary tract infections, urinary stones, or any other urological condition, acute renal failure and dehydration, patients with end-stage renal disease, whether or not on hemodialysis, and patients with malignancies were also excluded. Exclusion was based on medical history, physical examination, and basic laboratory tests that revealed any of the exclusion criteria.

After receiving Institutional Review Board (IRB) approval, the study was approved by the internal medicine department of Zagazig University Hospitals. The current study followed all the procedures outlined in the current iteration of the Helsinki Declaration. All participants were notified about the study's different aspects, and they were only enrolled after signing a consent form.

History Taking from all study participants was done, as well as thorough clinical examination. A complete general, cardiovascular, pulmonary, abdominal, and neurological

examination was performed. Routine investigations were performed in accordance with the Zagazig University Hospital's clinical pathology laboratory policy. Complete blood count (CBC), fasting blood glucose and glycated haemoglobin (Hb A1c), liver function tests, erythrocyte sedimentation rate (ESR), serum creatinine and blood urea, and estimation of glomerular filtration rate (eGFR), iron study to rule out iron overload (serum iron, total iron binding capacity, and transferrin saturation).Antinuclear antibodies (ANA) and Anti-double strandeddeoxyribonucleic acid (dsDNA) antibodies were evaluated (titre and pattern) by indirect immunofluorescence (Inova Diagnostics, USA). Assessment of complement (C3 and C4) were measured by turbidimetry on Cobas 6000 analyser (Roche Diagnostics, Switzerland).Commercial ELISA kits were used to test ferritin levels in the urine and serumaccording to manufacturer instructions (Sunred Biotechnology, China).To calculate the urine ferritin creatinine ratio, urinary ferritin was normalised by urinary creatinine (UFCR). Ultrasonography of the abdomen and pelvis has been used when appropriate. The National Kidney Foundation's MDRD equation was used to compute estimated GFR (eGFR) for all study participants.

Assessment Of The SLE Disease Activity

The systemic lupus erythematosus disease activity index (SLEDAI) was used to assess disease activity, which is a valid and reliable model among experienced practitioners for evaluation of disease activity in patients with SLE[4].

STATISTICAL ANALYSIS

The collected data was analysedusing MedCalcStatistical Software version 18.9.1 (MedCalcSoftware bvba, Ostend, Belgium;http://www.medcalc.org; 2018).Appropriate analysis was performed for each parameter based on the type of data obtained.

Continuous variables were expressed as mean ± SD and categorical variables was expressed as number (percentage). Continuous data was checked for normal Gaussiandistribution. All normally distributed data were analyzed using independent student (t) test. Non normally distributed data were analyzed using the Mann Whitney U (MW) test. One way ANOVA was used to compare normally distributed variables between three groups if data are normally distributed. Kruskal–Wallis H test (KW) was used to compare non normally distributed data between more than two groups.Percentage of categorical variables was compared using the chi square (X2) test.The spearman rank correlation and Pearson correlation coefficient were calculated to assess correlation between UFCR and other parameters.**Receiver operating characteristic (ROC) curve** analysis was used to identify the utility of **UFCR** for prediction of SLE with maximum sensitivity and specificity of generated cut off values.A p value < 0.05 was consideredstatistically significant (S), and if $p \geq 0.05$ then was considered non significant (NS).

Results:

In the current study, we enrolled fifty-four participants [three males (5.6%) and 53 females (94.4%)]. The average age of the participants in the study was 31.3 ± 6.35 years. Patients with lupus mean age was 30.81 ± 6.52 years including one male and 35 females.Comparison of laboratory data among the studied groups are summarized in table 1.

The mean ANA titer of SLE patients ranged between (0.1 – 112.3) with mean of 24.36 ±23.85, and antidsDNA titers ranged between (10 – 193.6) with mean of 75.50 ± 50.66. After categorization of SLE patients into two groups according to presence or absence of LN, the immunological profile and inflammatory markers are shown in table 2.
As regard the SLE activity assessed by SLEDAI score, it was ranged between (4 – 36) with mean SLEDAI score of 14.37 ± 8.09. As regard the presenting feature in SLE patients, hypertension was evident in nine patients (25%), convulsions in six patients (16.67)%, proteinuria in 17 patients (47.22%), headache in eight patients (22.22%), skin rash in 20 patients (55.56%), hair loss in 19 patients (52.78%), fever in six patients (16.67%), arthritis in 13 patients (36.11%), myositis and hematuria in four patients (11.11%).
After estimation of GFR utilizing CKD-EPI formula, eGFR ranged from 16 – 142 mL/min with mean of 88.87 ± 22.1 mL/min. As regard comparison of renal function between lupus patients with or without LN it is summarized in table 3.
There was statistical significant difference in urinary ferritin between SLE patients (M=58.22, SD=26.81) and control group (M=23.13, SD=13.43); t(52)=-5.22 , p<0.001. After correction with urinary creatinine, there was statistical significant difference in UFCR between SLE patients (M=7.27, SD=4.72) and control group (M=0.6, SD=0.36); t(52)=-5.96 , p<0.0001. As regard other parameters concerning iron metabolism it is summarized in table 4
The correlation between UFCR and other study parameters weretested, there was positive correlation between UFCR and SLEDAI in SLE patients (n= 36,r = 0.62, *P* < 0.001), as regard correlation between UFCR and other study parameters see table 5.
For the detection of SLE,at a best cut off value of UFCR (>1.33 mg/mol), the sensitivity and specificity were 91.7% and 88.9%, respectively, with an AUC of 90.7% (figure 1).

Table (1): Comparison of laboratory data among the studied groups:

Variable		SLE with or without LN (n=36)	Control Group (n=18)	Test	P
Hb: (gm/dl)	*Mean ± SD*	11.47±1.70	11.81±1.13	t 0.75	0.46 NS
Platelets: ($x10^3/mm^3$)	*Mean ± SD*	248.25±81.06	228.07±65	t -0.92	0.36 NS
WBCs: ($x10^3/mm^3$)	*Median(Range)*	6.65(2.8-19.6)	6.72(4.37-10.53)	MW 0.3	0.76 NS
T. protein: (gm/dl)	*Mean ± SD*	7.24±0.97	7.51±0.90	t 0.98	0.33 NS
Albumin: (gm/dl)	*Mean ± SD*	3.87±0.61	4.36±0.43	**t 3.06**	**0.004 S**

t= Independent sample t Test
MW= Mann Whitney Test
NS: non significant (P>0.05)
S: Significant (p<0.05)

Table (2): Comparison of immunological profile and inflammatory markers among the studied cases group:

Variable		Group I (SLE without LN) (*n*=18)		Group II (SLE with LN) (n=18)		Test	P
		No	%	No	%		
ANA	-	2	11.1	0	0	χ^2	
	+	4	22.2	2	11.1	3.29	0.35
	++	8	44.4	10	55.6		NS
	+++	4	22.2	6	33.3		
Titer:	Median (Range)	15.85(0.2-76)		26.95(5.3-112.3)		MW 1.52	0.13 NS
dsDNA	-	5	27.8	0	0	χ^2	
	+	3	16.7	2	11.1	6.59	0.09
	++	7	38.9	11	61.1		NS
	+++	3	16.7	5	27.8		
Titer:	Median (Range)	75.5(10-193.6)		76(37-183)		MW 0.29	0.78 NS
C3:	Median (Range)	1.02(0.53-2.25)		0.80(0.40-1.16)		**MW 2.53**	**0.01***
C4:	Median (Range)	0.14(0.03-0.34)		0.11(0.04-0.24)		MW 1.48	0.14 NS
ESR: (mm)	Median (Range)	4.5(0.11-15)		14.8(5-82)		MW 0.30	0.76 NS

MW= Mann Whitney Test
X^2: Chi Squared Test
NS: non significant (P>0.05)
S: Significant (p<0.05)

Table (3): Comparison of renal function among the studied groups:

Variable		Group I (SLE without LN) (*n*=18)	Group II (SLE with LN) (n=18)	Test	P
Blood urea: (mg/dl)	*Median(Range)*	23.58(9.43-49.93)	42.43(16.5-168.2)	**MW 2.74**	**0.006 S**
eGFR: (ml/min/1.73m²)	*Median(Range)*	113(70-142)	79(16-90)	**MW 3.55**	**<0.001 S**
24 hour Protein: (mg/24h)	*Median(Range)*	112.95(47.5-446)	1280.7(71.4-6400)	**MW 4.4**	**<0.001 S**
S. Creatinine: (mg/dl)	*Median(Range)*	0.67(0.48-1.02)	0.9(0.60-3.49)	**MW 2.71**	**0.007 S**

MW= Mann Whitney Test
NS: non significant (P>0.05)
S: Significant (p<0.05)

Table (4): Comparison of iron test parameters among the studied groups:

Variable		SLE with or without LN (*n*=36)	Control Group (n=18)	Test	P
S.Iron: (µg/dl)	*Median (Range)*	40.4(11-214)	41(22-176)	MW 0.93	0.35 NS
TIBC: (µg/dl)	*Mean ± SD*	287.31±86.38	239.56±104.92	t -1.78	0.08 NS
TS: (%)	*Median (Range)*	12.5(2.5-117)	17.35(5-161.5)	MW 1.77	0.08 NS
U.Ferritin (ng/ml)	***Mean ± SD***	**58.22±26.81**	**23.13±13.43**	**t -5.22**	**<0.001 S**
S.Ferritin: (ng/ml)	*Median (Range)*	25.44(1.3-132.6)	27(2.6-150)	MW 0.31	0.76 NS
UFCR: (mg/mol)	***Mean ± SD***	**7.27±4.72**	**0.6±0.36**	**t -5.96**	**<0.001* S**

t= Independent sample t Test

MW= Mann Whitney Test
NS: non significant (P>0.05)
S: Significant (p<0.05)

Table (5): Correlation between different parameters and UFCR among SLE patients:

Variable	SLE patients with or without LN (n=36)	
	R	P
Age (years)	0.31	0.06 NS
ANA	0.08	0.63 NS
dsDNA	0.04	0.81 NS
C3	-0.14	0.43 NS
C4	-0.16	0.36 NS
SLEDAI	**0.62**	**<0.001 S**
Hb: (gm/dl)	0.08	0.63 NS
Platelets:($x10^3/mm^3$)	0.16	0.36 NS
WBCs:($x10^3/mm^3$)	0.32	0.06 NS
T. protein: (gm/dl)	-0.17	0.33 NS
Albumin: (gm/dl)	-0.31	0.06 NS
ESR: (mm)	0.07	0.70 NS
CRP: (mg/dl)	0.14	0.44 NS
S.Iron:(µg/dl)	0.10	0.58 NS
TIBC:(µg/dl)	0.22	0.20 NS
TS:(%)	-0.10	0.57 NS
S. Ferritin:(ng/ml)	**0.52**	**<0.001 S**
eGFR: (ml/min/1.73m^2)	**-0.36**	**0.03 S**
24 hour Protein:(mg/24h)	0.27	0.11 NS
S. Creatinine: (mg/dl)	**0.41**	**0.01 S**

r: Spearman’e correlation coefficient
NS: non significant (P>0.05)
S: Significant (p<0.05)

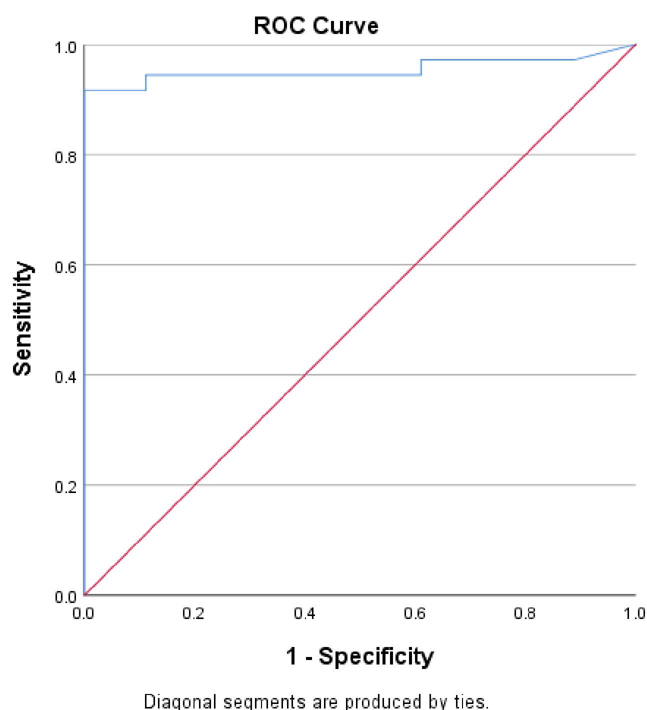

Figure (1): Roc curve for validity of UFCR in diagnosis of SLE among the studied groups.

Discussion

There was a statistical significant differences between groups as regard serum albumin.

As regard total protein and serum albumin, our results go in harmony with ***Sui et al.,*** [(7)] who found that serum albumin reflects disease activity in SLE patients with nephritis. Given this background, they hypothesized that nephrotic-range proteinuria, and decreased serum albumin levels may reflect the activity and severity of renal damage in patients with lupus nephritis.

This is explained by that serum albumin is routinely measured in patients with SLE as part of standard biochemical profiles. Lower than normal levels have been frequently reported in SLE. A low serum albumin level may be a result of increased albumin catabolism due to chronic inflammation and/or because of inadequate protein and caloric intake in patients with SLE. In addition, nephritis, a common manifestation of SLE, may lead to proteinuria, which in turn lowers serum albumin levels [(8)].

Regarding urinary ferritin creatinine ratio (UFCR), our results showed that there was a statistical significant difference between the studied groups with p-value: <0.001. It was significantly higher in the SLE group than the control group.

These findings was in agreement with ***Qi et al.,*** [(5)] who reported in their study that The UFCR level was significantly higher in severely (n = 28) or non-severely active SLE patients (n = 34) than that in HC (both P < 0.01). Also, they reported that the UFCR levels were significantly different among LN, SLE without nephritis and healthy control (P < 0.01). UFCR level in LN patients (n = 35) was significantly higher than that in lupus patients without LN (n = 27) (15.25 mg/mol (5.18, 33.25) vs. 2.01 mg/mol (0.69, 2.75), P < 0.01).

At least three factors contributing to the increased UFCR in LN. ***Firstly***, because ferritin cannot be filtered by the normal glomeruli, the glomerular damage should be the base of increased UFCR. ***Secondly***, many chronic kidney diseases, including LN, have tubulointerstitial lesions in the meantime, and tubular iron deposition has been found to be one of the causes of tubulointerstitial change in these diseases [(9).] Therefore, it is reasonable to presume that tubular iron deposition promotes ferritin formation and secretion in LN. ***In addition***, it has been demonstrated that activated macrophages increase in the kidneys of LN patients. These local macrophages may be important cells in charge for increased ferritin production and increased UFCR [(10).]

Regarding validity of UFCR in diagnosis of SLE, we found that at cut off point (> 1.33), UFCR has 91.7% sensitivity, 88.9% sensitivity, 94.2% PPV, 84.2% NPV and 90.7% accuracy. While at cut off point (>5.99).

Qi et al., [(5)] reported that the AUC of UFCR was 0.831, and a cutoff of 4.09 mg/mol yielded a sensitivity of 82.9% and specificity of 81.5% for diagnosing LN. And in the ROC curve of LN disease activity, the AUC of UFCR was 0.720, indicating that UFCR can be used as a reliable indicator for evaluating LN disease activity.

Conclusion:

UFCR level can be considered as a potential biomarker for SLE.

References:

1. **Ali A, Sayyed Z, Ameer MA, et al. (2018):** Systemic Lupus Erythematosus: An Overview of the Disease Pathology and Its Management.Cureus, 10; 9:e3288.
2. **Schwartz N, Michaelson JS and Putterman C. (2007):** Lipocalin-2, TWEAK, and other cytokines as urinary biomarkers for lupus nephritis.Ann N Y Acad Sci, 1109:265-74.
3. **Laufberger V. (1937):** Sur la cristallisation de la ferritine. Bulletin de la Societe de chimie biologique. 1937;19:1575–1582.
4. **Bradley JM, Le Brun NE and Moore G.R. (2016):** Ferritins: Furnishing proteins with iron. J Biol Inorg Chem, 21; 1:13–28.
5. **Qi L, Xu J, Yang C, et al. (2020):** Urinary ferritin creatinine ratio, a potential biomarker for lupus nephritis activity. Clin Rheumatol, DOI: 10.1007/s10067-020-05214-0.
6. **Verma N, Chakraverty J, Baweja P et al. (2017):** Extremely highferritinemia associated with hemophagocytic lymphohistiocytosis(HLH). Indian J Clin Biochem 32; 1:117–120.
7. **Sui M, Jia X, Yu C, Guo X, Liu X, (2014):** Relationship between hypoalbuminemia, hyperlipidemia and renal severity in patients with lupus nephritis: a prospective study. Cent Eur J Immunol. 2014;39(2):243-52.
8. **Yip J, Aghdassi E, Su J, Lou W, et al. (2010):** Serum Albumin as a Marker for Disease Activity in Patients with Systemic Lupus Erythematosus.J Rheumatol 2010; 37; 1667-1672
9. **van Raaij S, van Swelm R, Bouman K et al. (2018):** Tubular iron deposition and iron handling proteins in human healthy kidney and chronic kidney disease[J]. Sci Rep 8(1):9353
10. **Wang Y, Li MH, Zhang Y et al. (2019):** Relationship between podocyte injury and macrophage infiltration in renal tissues of patients with lupus nephritis] [J]. Beijing Da Xue Xue Bao Yi Xue Ban 51(4):723–727