The Egyptian Journal of Hospital Medicine (October 2022) Vol. 89 (2), Page 7684- 7695

Beta 2 Microglobulin and Cystatin C in Systemic Lupus Erythematosus Patients: Correlation with Disease Activity

Abdullah Badreddin Youssef[1]*, Atif Ibrahim Elghaweet[1], Sherin Mohamed Abd El-Aziz[2], Abeer Abd El-Hamid Fikry[1]
Departments of [1]Physical Medicine, Rheumatology & Rehabilitation and
[2]Clinical Pathology, Faculty of Medicine, Mansoura University, Egypt
***Corresponding author:** Abdullah Badreddin Youssef, **Mobile:** (+20) 01022118568, **E-Mail:** risinigagain@yahoo.com

ABSTRACT
Background: Systemic lupus erythematosus (SLE) is a serologically & clinically heterogeneous disorder that is associated with abnormal immune response. β2MG is a low-molecular-weight-protein and is mainly released from immune-related cells including activated T- and B-cells and macrophages. Cystatin C is a low molecular cysteine protease inhibitor secreted by almost all nucleated cells in humans.
Objective: The aim of this work was to assess serum β2MG and Cystatin C levels among SLE patients and control subjects and assess their possible correlation with disease activity.
Patients and methods: This case control study was conducted on 40 SLE patients and 40 apparently healthy subjects. The SLEDAI-2K was utilized to asses disease activity. Score was graded as: < 3 mild, 3–6 moderate, and > 6 severe.
Results: The mean serum β2MG level and Cystatin C level were statistically significantly greater among SLE patients compared with in healthy controls. There was significant correlation between serum β2MG and Cystatin C concentrations and SLEDAI-2k disease activity score. The Receiver Operating Characteristics curve for serum β2MG and serum Cystatin C utilized to differentiate between active and non-active SLE patients with the best detected cut off point were 6.65 and 1.13 yielding sensitivity of 78.3% and 73.9% respectively, specificity of 70.6% and 35.3%, respectively.
Conclusion: Serum β2MG concentrations are significantly elevated in SLE patients in comparison with healthy subjects. The sensitivity, specificity and accuracy of β2MG were higher than that of Cystatin C in identifying SLE activity and differentiation between SLE patients and controls.
Keywords: Systemic lupus erythematosus, anti-ds DNA, Beta 2 microglobulin, Cystatin C.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a serologically & clinically heterogeneous disorder which is associated with abnormal immune response. It is a multiorgan disease characterized by the production of multiple antinuclear antibodies and presence of immune complexes in the affected organs **(1).**

SLE manifestations are widely variable and many methods are available to measure disease activity. One of the most widely used instruments is Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K). Other methods include serum anti-double-stranded DNA antibodies (anti-ds DNA), C3 and C4 complement components, anti-C1q antibody, Systemic Lupus Activity Measure (SLAM), British Isles Lupus Assessment Group (BILAG), European Consensus Lupus Activity Measurement (ECLAM) **(2).** CRP is a non-specific biomarker of several inflammatory conditions, however it might be used as a direct scale of disease or to predict prognosis of autoimmune disorders **(3).** Beta 2 microglobulin (β2MG) is a light-chain subunit of class I human leukocyte antigen (HLA) which is present on the cell membranes of all nucleated cells **(4).**

The relationship between serum β2MG level and disease activity has not fully explained. Some studies failed to report such an association because of its limited utilization in patients with kidney affection characterized by decreased GFR **(5).** Few studies showed positive association between β2MG blood concentration and SLE activity **(6).** Other studies demonstrated that β2MG can serve as a reliable biomarker of kidney function in SLE patients as it is correlated negatively with eGFR and positively with serum creatinine **(7)**.

Cystatin C is a reversible inhibitor of cysteine proteinases which is released by the majority of cells **(8).** A previous study found that serum Cystatin C correlates with SLE disease activity **(9).** It is mainly valuable for the determination of mild kidney affection, a common complication of SLE **(10).** Cystatin C can serve as a reliable biomarker of kidney function among SLE patients as it is correlated negatively with eGFR and positively with serum creatinine **(11).**

The aim of the current work was to evaluate serum β2MG and Cystatin C levels among SLE patients and control subjects and assessed their possible correlation with disease activity.

PATIENTS AND METHODS

Study Design

The current work was included 40 SLE patients recruited from outpatient clinic of Physical Medicine, Rheumatology and Rehabilitation Department, Mansoura University Hospitals from September 14, 2021 to April 15, 2022.

Received: 19/7/2022
Accepted: 21/9/2022\

Patients' Group:

This study included 40 patients aged 18 years or more, the selected SLE patients fulfilled at least score of 10 or more of ACR/EULAR 2019 revised classification criteria for SLE (**Figure 1**) [(12)].

However, we excluded patients with infection, tuberculosis, with any type of malignancy or metastasis, with chronic kidney disease associated with other Rheumatic autoimmune diseases (e.g. Rheumatoid Arthritis, systemic sclerosis). Also, we excluded patients with history of drug inducing lupus, or pregnant and lactating females. The age range for the participants was 18 - 52 years and they were 32 females and 8 males. Disease duration was in the range of 1-8 years.

Control group:

A total of 40 healthy age- and sex-matched persons to SLE patients were included as control group.

Methods:

All the eligible patients were subjected to the history taking including personal history (name, age, sex, marital state, residency, occupation, and any special habit of medical significance), complaints (taken in patient's own words), present history (analysis of patient's complaint, presentation of disease, mode and date of onset, course and duration of disease, and presenting feature), constitutional manifestations (Fever, weight loss, fatigue), mucocutaneous manifestations (ulcers, skin rash, falling of hair, photosensitivity, dry mouth, and alopecia), ocular manifestation (redness, dryness, diminution of vision), musculoskeletal symptoms (stiffness, swelling, muscle pain, bony aches), cardiovascular symptoms (cough, hemoptysis, dyspnea, chest pain and history suggestive of venous or arterial thrombosis), respiratory symptoms (Cough, Dyspnea, hemoptysis, cyanosis and wheezes), neuropsychiatric symptoms (anxiety, seizures), gastrointestinal symptoms (anorexia, vomiting, epigastric pain, dysphagia, regurgitation or heart burn, hematemesis or melena, severe abdominal pain, bowel disturbance), urinary symptoms (dysuria, hematuria, renal colic) and menstrual troubles (amenorrhea, menorrhagia, menstrual irregularities).

We also asked the patients about their therapeutic history (drugs used, duration of therapy and dosage, continuity and cause of stoppage), past history (diseases, trauma or operation, drug allergy) and family history (history of similar conditions in the family or other autoimmune diseases)

Complete physical examination was performed to all patients and it included included; BMI, vital signs, facies features (cushingoid facies), body built, Scalp (alopecia), eye (puffiness, jaundice, pallor), mouth and pharynx (ulceration, pallor, central cyanosis), extremities (peripheral cyanosis, raynaud's phenomenon, edema), joints (range of motion as regards instability, pain on movement, crepitus and protective muscle spasm).

Entry criterion

Antinuclear antibodies (ANA) at a titer of ≥1:80 on HEp-2 cells or an equivalent positive test (ever)

↓

If absent, do not classify as SLE
If present, apply additive criteria

↓

Additive criteria

Do not count a criterion if there is a more likely explanation than SLE.
Occurrence of a criterion on at least one occasion is sufficient.
SLE classification requires at least one clinical criterion and ≥10 points.
Criteria need not occur simultaneously.
Within each domain, only the highest weighted criterion is counted toward the total score§.

Clinical domains and criteria	Weight	Immunology domains and criteria	Weight
Constitutional		***Antiphospholipid antibodies***	
Fever	2	Anti-cardiolipin antibodies OR	
Hematologic		Anti-β2GP1 antibodies OR	
Leukopenia	3	Lupus anticoagulant	2
Thrombocytopenia	4	***Complement proteins***	
Autoimmune hemolysis	4	Low C3 OR low C4	3
Neuropsychiatric		Low C3 AND low C4	4
Delirium	2	***SLE-specific antibodies***	
Psychosis	3	Anti-dsDNA antibody* OR	
Seizure	5	Anti-Smith antibody	6
Mucocutaneous			
Non-scarring alopecia	2		
Oral ulcers	2		
Subacute cutaneous OR discoid lupus	4		
Acute cutaneous lupus	6		
Serosal			
Pleural or pericardial effusion	5		
Acute pericarditis	6		
Musculoskeletal			
Joint involvement	6		
Renal			
Proteinuria >0.5g/24h	4		
Renal biopsy Class II or V lupus nephritis	8		
Renal biopsy Class III or IV lupus nephritis	10		

Total score:

↓

Classify as Systemic Lupus Erythematosus with a score of 10 or more if entry criterion fulfilled.

Figure (1): ACR/EULAR 2019 revised classification criteria for SLE [(12)].

Assessment of Disease activity for SLE patients:

The SLEDAI-2K (**Figure 2**) was calculated after evaluating of neuropsychiatric features (seizures, psychosis, organic brain syndrome, lupus headache), visual disturbances, cerebrovascular accidents, vasculitis, arthritis, myositis, kidney features (hematuria, proteinuria, pyuria), rash, hair loss, oral ulcer, serositis (pleurisy, pericarditis), reduced complement, enhanced DNA binding, fever, hematological features (thrombocytopenia, leukopenia) [(13)]. SLEDAI-2K is based on the existence of 24 descriptors in 9 organs over the previous 4 weeks. These descriptors were described as present or absent. Each descriptor had a score and the total score of SLEDAI-2K was the sum of scores of all 24 descriptors. The total SLEDAI-2K score ranges from 0 to 105, with greater scores indicating higher activity [(14)]. SLEDAI-2Kscore was graded as: score <3 mild disease, 3–6 moderate disease, and any SLEDAI-2K score >6 severe disease [(15)].

Laboratory investigations: The investigations included - CBC, ESR, CRP, Blood urea, serum creatinine, protein/creatinine ratio, Anti-ds DNA, anti-nuclear antibodies (ANA), C3, C4, Serum β2MG, and Serum Cystatin C.

Sample collection: Six milliliters venous blood sample was withdrawn from each subject by sterile venipuncture. One ml was delivered into EDTA blood for complete blood count (CBC). Two ml was delivered into citrated tube for ESR level. The remaining 3ml were delivered into plain tube and left for clotting then, after centrifugation at 3000 rpm for 15 minutes; serum was separated and divided into 2 aliquots. The first aliquot was used for detection of CRP, ANA, anti-double strands, kidney functions.

Detection of β2MG Using ELISA kits (mg/L): Using ELISA kits were purchased from Shanghai Sun Red Biological Technology Co., Ltd. This kit was utilized to quantify β2MG in serum, plasma, and other related tissue Liquid.

Test principle of β2MG**:** The kit used a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to measure β2MG level in human samples. β2MG was added to monoclonal antibody Enzyme well that was pre-coated with Human β2MG monoclonal antibody. Then incubation was done and β2MG antibodies labeled with biotin were added, and combined with Streptavidin-HRP forming immune complex. This was followed by incubation and washing to remove any uncombined enzyme. After that, Chromogen Solution A, B was added and the colour changed into bluish and at the effect of acid, the colour changed into yellowish. The chroma of colour and β2MG level were positively correlated.

Detection of Cystatin C by (mg/L) level: Using ELISA. Kits were purchased from Shanghai Sun Red Biological Technology Co., Ltd. This kit was utilized to quantify Cystatin C in serum, plasma, and other related tissue Liquid.

Test principle of Cystatin C: The kit used a double-antibody sandwich ELISA to measure Cystatin C level in human samples. Cystatin C was added to monoclonal antibody Enzyme well that was pre-coated with Human Cystatin C monoclonal antibody. Then incubation was done and Cystatin C antibodies labeled with biotin were added, and combined with Streptavidin-HRP forming immune complex. This was followed by incubation and washing to remove any uncombined enzyme. After that, Chromogen Solution A, B was added and the colour changed into bluish and at the effect of acid, the colour changed into yellowish. The chroma of colour and the concretum anion of Cystatin C were positively correlated.

Ethical consent:

The Ethical Institutional Review Board at Mansoura University approved the study. After explaining our research objectives, written informed consent was obtained from all study participants. This study was conducted in compliance with the code of ethics of the world medical association (Declaration of Helsinki) for human subjects.

Statistical analysis:

Data were analyzed by Statistical Package of Social Sciences (SPSS version 20). Two types of statistical analysis were conducted; descriptive statistics that included estimates for summarizing the continuous data as mean and standard deviation (SD) or median and range for skewed data. Frequency with percentage (%) was used for presenting qualitative data and analytical or inferential statistics that included Pearson Chi-square ($\chi 2$) test, independent samples t-test (t test) and Mann-Whitney U-test (Z test). The results were considered significant if P value is ≤ 0.05 and highly significant if the P value <0.001.

RESULTS

This was a case-control study conducted on 40 SLE patients and matched age and sex 40 apparent subjects acting as controls. There were no statistically significant differences between both groups as regards age, gender and BMI (**Table 1**).

Table (1): Demographics and BMI of SLE patients and controls

Variable		SLE patients (N=40)		Controls (N=40)		Test of significance
Age (years)		33.90 ± 5.26		35.90 ± 5.26		t= -1.702 P = 0.093
Gender	Male	8	20%	14	35%	$\chi 2$= 2.257 P= 0.133
	Female	32	80%	26	65%	
BMI (years)		27.73 ± 4.17		27.98 ± 4.69		t= -0.964 P = 0.572

t= independent samples t-test. $\chi 2$= Chi-square test.

In SLE patients, arthralgia and arthritis were the most common clinical finding in 30 (75%) and 28 (70%), respectively. Other manifestations include renal manifestations in 67.5%, malar rash in 62.5%, alopecia in 52.5%, oral ulcers in 25%, Raynaud's disease in 25%, photosensitivity in 22.5%, rash in 20%, vomiting in 15%, fever in 12.5%, pleuritis in 12.5%, fatigue in 12.5%, nausea in 12.5% discoid rash in 10%, headache in 10% and vasculitis in 2.5%.

Mean hemoglobin concentration, mean hematocrit value, and platelets count in the SLE group were significantly lower as compared to controls. Other CBC parameters didn't show a significant difference between both groups.

The mean ESR in the SLE group was significantly higher in comparison with controls. There was significant difference in the CRP level between both groups. The mean C3 and mean C4 in the SLE group were significantly lower in comparison with controls. ANA titer and anti-ds DNA titer in the SLE group were significantly higher in comparison with controls. Table 2 compares blood picture and immunological parameters between SLE patients and controls.

Table (2): Blood picture and Immunological parameters of SLE patients and controls.

Variable	SLE patients (N=40)	Controls (N=40)	Test of Significance	P value
Hemoglobin (gm/dl)	9.22 ± 2.24	10.41 ± 1.60	t= - 2.735	0.004*
RBC (10^6/ml)	3.86 ± 0.93	4.17 ± 0.75	t= - 1.660	0.101
Hematocrit (%)	29.51 ± 7.16	32.59 ± 6.38	t= - 2.027	0.046*
MCV (femtoliters/cell)	78.49 ± 7.25	78.77 ± 6.76	t= - 0.176	0.861
MCH (picograms/cell)	25.07 ± 3.06	25.12 ± 2.99	t= - 0.069	0.945
MCHC (gm/dl)	31.75 ± 2.99	31.02 ± 2.13	t= 0.248	0.722
WBCs (10^3/ml)	6.4 ± 1.21	7.0 ± 1.32	z= - 1.097	0.273
Neutrophils (10^3/ml)	1.0 ± 0.025	56.29 ± 11.37	t= 1.206	0.230
Lymphocytes (10^3/ml)	0.5 ± 0.003	31.24 ± 7.34	t= - 1.053	0.360
Neutrophil/lymphocyte ratio	2.0±0.28	1.80±0.43	t=1.25	0.415
PLTs(10^3/ml)	204 ± 48.31	312.5 ± 7.51	z= - 2.926	0.003*
ESR (mm/h)	74.85 ± 17.16	9.55 ± 1.69	t= 30.884	< 0.001
CRP (mg/dl)	8.0 ± 1.8	0	z= 2.15	0.002
C3(mg/dl)	65.28 ± 4.32	133.13 ± 21.55	t= -17.325	< 0.001
C4(mg/dl)	40.56 ± 10.11	73.27 ± 15.94	t= -9.414	< 0.001
ANA	138.0 ± 32.4	0	z= 5.69	< 0.001
Anti-ds DNA antibodies	36 ± 8.32	0	z= 4.25	< 0.001

*: statistically significant (P<0.05)

Table 3 summarizes disease activity score of SLE patients.

Table (3): Disease activity score of SLE patients.

Variable		SLE patients (N=40)
SLEDAI-2k	Mean ± SD	4.92 ±4.51
	Median (range)	4 (0 -12)
SLEDAI-2k categories		
No activity		9(22.5%)
Mild		8(20.0%)
Moderate		20 (50%)
Severe		3 (7.5%)

Table 4 compares kidney function tests between SLE patients and controls.

Table (4): Kidney function tests of SLE patients and controls

Variable	SLE patients (N=40)	Controls (N=40)	Test of Significance	P value
Creatinine (mg/dl)	1.3 ± 0.30	1.1 ± 0.21	z= - 4.203	< 0.001*
Protein/creatinine ratio	2.61 ± 0.5	0.19 ± 0.031	z= - 7.186	< 0.001*
Protein in urine (mg/24 hours)	291 ± 7.0	94 ± 21.3	z= - 7.743	< 0.001*
GFR (mL/min/1.73m^2)	47 ± 11.12	105.1 ± 23.31	z= - 7.54	< 0.001*

P: probability. Continuous data expressed as median (min-max). Z= Mann-Whitney test. *: statistically significant (P<0.05).

Table 5 compares serum β2MG **and serum Cystatin C** between SLE patients and controls.

Table (5): Comparison between SLE patients and control as regard serum β2MG - (mg/L) and serum Cystatin C (mg/L).

Variable	SLE patients (N=40)	Controls (N=40)	Test of Significance	P value
Serum β2MG (mg/L)	6.72 ± 1.05	2.79 ± 0.26	t= 23.030	< 0.001*
Serum Cystatin (mg/L)	1.05 ± 0.13	0.88 ± 0.07	t= 7.608	< 0.001*

P: probability. Continuous data expressed as mean ± SD. t= independent samples t-test. *: statistically significant (P< 0.05).

Significant positive correlations existed between serum β2MG and serum creatinine, ESR, ANA, anti-ds DNA, SLEDAI-2K and Cystatin C. Also, there were significant negative correlation between serum β2MG with GFR, C3 and C4. Other variables didn't show a statistically significant correlation with Serum β2MG. There were significant positive correlations between serum Cystatin and serum creatinine, ESR, ANA, anti-ds DNA, SELDAI-2K and β2MG. Also, there were significant negative correlation between serum Cystatin with GFR, C3 and C4. Other variables didn't show a statistically significant correlation with serum Cystatin (**Table 6**).

Table (6): Correlations between serum β2MG, Cystatin C and laboratory data in SLE patients.

Variable		Serum β2MG	Cystatin
HGB	r	0.049	0.027
	P	0.763	0.869
RBCs	r	-0.141	0.094
	P	0.387	0.563
Hct	r	-0.060	-0.039
	P	0.715	0.813
MCV	r	-0.093	-0.092
	P	0.568	0.574
MCH	r	0.079	-0.264
	P	0.626	0.100
MCHC	r	0.344	0.073
	P	0.112	0.652
TLC	r	-0.274	-0.129
	P	0.087	0.428
Neutrophil	r	0.153	0.026
	P	0.346	0.871
Lymphocytes	r	-0.141	-0.210
	P	0.385	0.193
Platelets	r	0.141	0.143
	P	0.385	0.378
Serum creatinine	r	**0.421**	**0.523**
	P	**0.04***	**0.03***
Protein/ creatinine ratio	r	0.178	-0.100
	P	0.272	0.540
24-h protein in urine	r	0.107	-0.041
	P	0.512	0.801
GFR	r	**-0.514**	**-0.565**
	P	**0.03***	**0.02***
TSB	r	0.091	0.001
	P	0.575	0.993
DSB	r	0.032	0.075
	P	0.847	0.645
Serum albumin	r	0.149	-0.012
	P	0.358	0.943
ALT	r	-0.265	0.185
	P	0.098	0.252
AST	r	-0.253	0.149
	P	0.115	0.360
ESR	r	**0.786**	**0.766**
	P	**< 0.001***	**< 0.001***
CRP	r	0.248	0.304
	P	0.376	0.158
C3	r	**-0.815**	**-0.740**
	P	**< 0.001***	**< 0.001***
C4	r	**-0.764**	**-0.769**
	P	**< 0.001***	**< 0.001***
ANA	r	**0.736**	**0.816**
	P	**< 0.001***	**< 0.001***
Anti-ds DNA	r	**0.826**	**0.828**
	P	**< 0.001***	**< 0.001***
SLEDAI_2K	r	**0.755**	**0.436**
	P	**< 0.001***	**0.049***
Cystatin	r	**0.904**	
	P	**< 0.001***	
β2MG	r		**0.904**
	P		**< 0.001***

r_s: Spearman's correlation. P: Probability. *: Statistically significant (P ≤0.05).

Receiver operating characteristics curve for serum β2MG (mg/L) is used to differentiate between active and non-active SLE patients with the best detected cut off point is 6.65 yielding sensitivity of 78.3%, specificity of 70.6% and total accuracy 75%.

Receiver operating characteristics curve for serum β2MG is used to differentiate between SLE patients and control group, found that area under ROC curve for this marker is excellent (AUC=0.957) with the best cutoff point is > 4.89 mg/l with the group higher than this value are SLE patients and group with serum β2MG (mg/L) < 4.89 are control group with 95% sensitivity, 97.5% specificity, 95% PPV, 92.5% NPV and 95% accuracy. Receiver operating characteristics curve for serum Cystatin (mg/L) is used to differentiate between active and non-active SLE patients with the best detected cut off point is 1.13 yielding sensitivity of 73.9%, specificity of 35.3% and total accuracy 57.5%.

Receiver operating characteristics curve for serum Cystatin (mg/L) is used to differentiate between SLE patients and control group, found that area under ROC curve for this marker is excellent (AUC=0.869) with the best cutoff point is > 0.994 mg/l with the group higher than this value are SLE patients and group with serum β2MG (mg/L) < 0.994 are control group with 87.5% sensitivity, 62.5% specificity, 75% PPV, 80% NPV and 72.5% accuracy (**Table 7; Figures 3, 4, 5, and 6**).

Table (7): Predictive value of serum β2MG (mg/L) and Serum Cystatin C in identifying SLE disease activity and identifying SLE patients.

Diagnostic criteria	Serum β2MG (mg/L)		Serum Cystatin C	
	identifying SLE disease activity	identifying SLE patients	identifying SLE disease activity	identifying SLE patients
AUC	0.708	0.957	0.579	0.869
Cut off point	6.65	> 4.89	1.13	> 0.994
P value	**0.026***	**< 0.001***	**0.396**	**< 0.001***
Sensitivity	78.3	**95 %**	73.9	**87.5 %**
Specificity	70.6	**97.5 %**	35.3	**62.5 %**
PPV	**78.3**	**95 %**	**60.7**	**75 %**
NPV	**70.6**	**92.5 %**	**50.0**	**80 %**
Accuracy	**75.0**	**95 %**	**57.5**	**72.5 %**

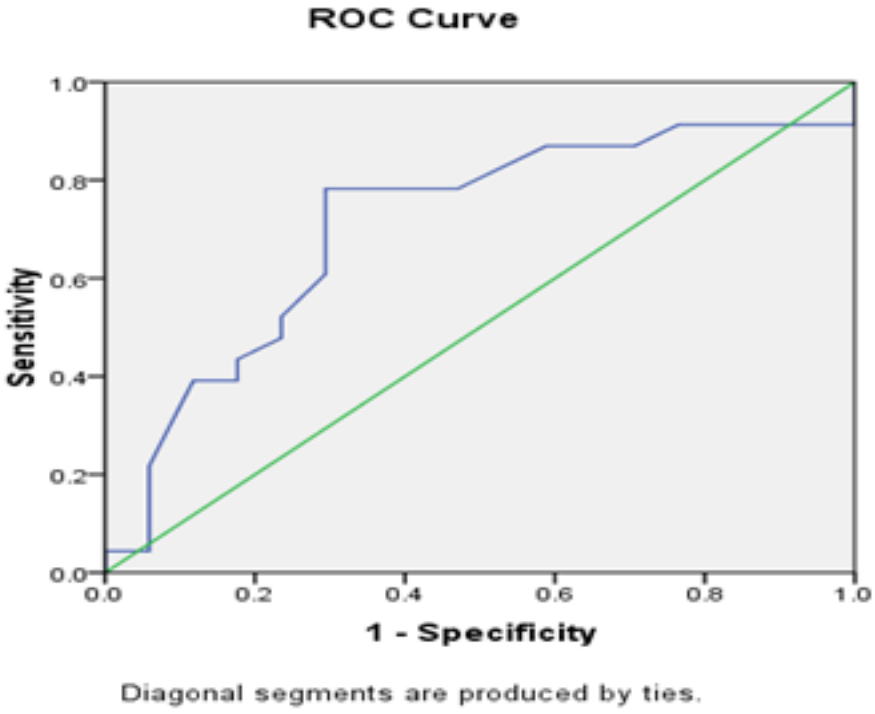

Figure (3): ROC curve analysis of serum β2MG (mg/L) in identifying SLE disease activity.

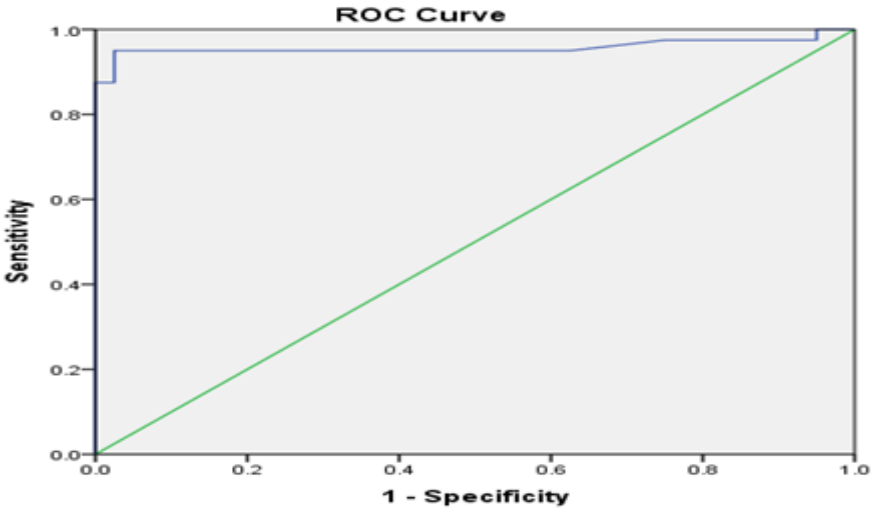

Figure (4): ROC curve analysis of serum β2MG (mg/L) in identifying SLE patients.

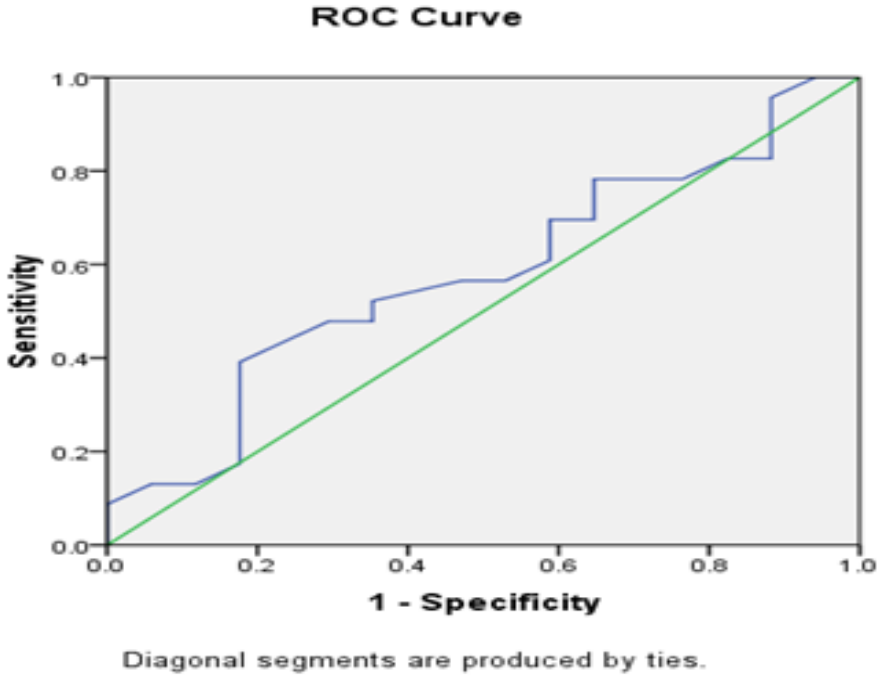

Figure (5): ROC curve analysis of Serum Cystatin C in identifying SLE disease activity.

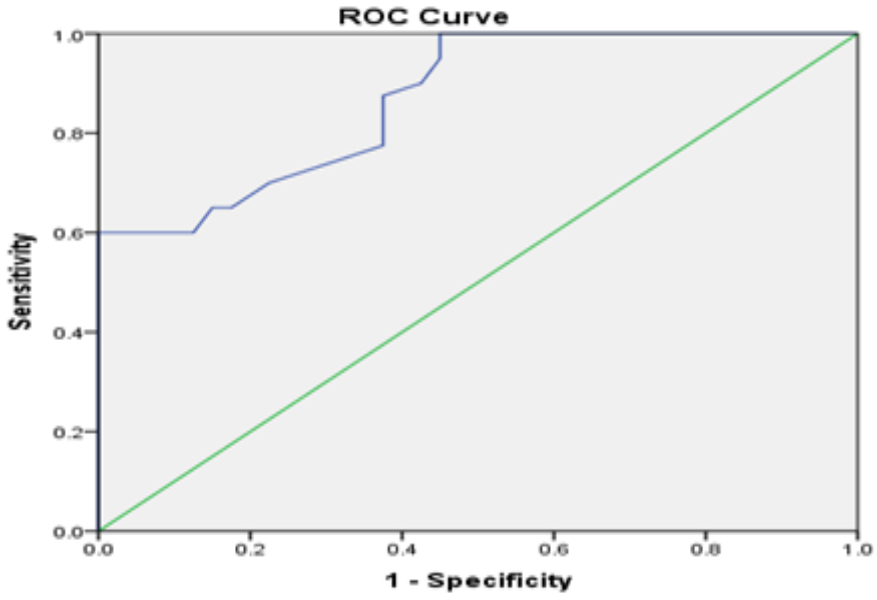

Figure (6): ROC curve analysis of serum Cystatin C (mg/L) in identifying SLE patients.

DISCUSSION

SLE is a chronic autoimmune disorder with alternating periods of exacerbations and remissions. Assessment of SLE activity is significant to select the proper treatment. Current blood markers do not have sufficient sensitivity or specificity in relation to alterations in disease activity. Thus, there is an ongoing search for clinically valuable biomarkers of activity [16].

Several studies [2,7,17,18] have revealed that β2MG is linked to disease activity. On the contrary, **Wakabayashi *et al.*** [19] demonstrated no significant association between serum β2MG level and SLE disease activity.

Lertnawapan *et al.* [20] and **Chew *et al.*** [8] have shown that Cystatin C is correlated with SLE disease activity**.** On contrary **El-Shafey *et al.*** [21] and **Garcia-Garcia *et al.*** [22] reported no significant correlation between Cystatin C level and SLE disease activity.

The aim of the current work was to assess serum β2MG and Cystatin C levels among SLE cases and control persons and assess their possible correlation with disease activity. This was a case-control study conducted on 40 SLE cases and matched age and sex 40 apparent healthy subjects acting as controls. All of the previously conducted studies were mainly emphasized on β2MG or Cystatin C separately. However, the current study also evaluated both of which to compare between their reliability.

In this study, the mean serum β2MG value was statistically significantly greater among SLE cases as compared with healthy controls (P <0.001).

Our finding was consistent with those of **Kim *et al.*** [5] who have demonstrated that; β2MG values of SLE cases were higher than control persons (P <0.001). They concluded that measurement of β2MG appear to be a valuable biomarker to assess disease activity of SLE.

In the same line, **Abd-Elbaky *et al.*** [16] have found a significant elevation of serum β2MG value among SLE patients in active and inactive groups (mean 6.77 ± 1.83 and 2.59 ± 0.43 mg/L), in comparison to control persons (0.82 ± 0.20mg/L, P=0.000), and its level increased in the active SLE group (mean 6.77 ± 1.83 mg/L) in comparison to in the inactive group (mean 2.59 ± 0.43 mg/L).

Regarding association between serum β2MG, clinical and hematological features of SLE cases, the current study demonstrated no significant associations between serum β2MG, clinical and hematological features in SLE cases.

Żychowska *et al.* [2] have displayed that β2MG levels was significantly greater in those with arthritis and/or myositis (P =0.005) and vasculitis (P =0.005). But our results were consistent with those of **Skare *et al.*** [6] who have demonstrated that there was no significant association between serum β2MG and hemoglobin.

Concerning association between serum β2MG and immunological parameters of SLE (ESR, CRP, C3, C4, ANA, anti-ds DNA), the current study demonstrated that there were significant positive correlation between serum β2MG and ESR (P <0.001), ANA (P <0.001) and anti-ds DNA (P <0.001). Also, there were significant negative correlation between serum β2MG with C3 (P <0.001) and C4 (P <0.001).

Żychowska *et al.* [2] have reported that; there were a significant correlation between β2MG level and anti-dsDNA titer (r = 0.3, P <0.05), and C4 component serum level (r = -0.3; P <0.05). but there was no significant association between β2MG and C3 component, but **Aghdashi *et al.*** [18] have demonstrated that no significant correlation were seen between serum β2MG value and anti-dsDNA antibodies, C3 and C4 but there are significant correlations between the level of β2MG and, ESR, and CRP (P <0.05).

In the current study the Receiver Operating Characteristics curve for serum β2MG (mg/L) is used to differentiate between active and non-active SLE patients with the best detected cut off point is 6.65 yielding sensitivity of 78.3%, specificity of 70.6% and accuracy 75%.

Tony *et al.* [1] performed a study on 40 SLE female cases and 40 controls which revealed that the serum β2MG had high specificity (100%) but low sensitivity (60%) for assessing disease activity in SLE patients.

A study made by **Liu *et al.*** [7] showed the predictive value of serum β2MG values for SLE disease activity was evaluated and they concluded that, β2MG had high sensitivity and specificity in predicting SLE, SLE disease activity and eGFR.

In this current study the Receiver Operating Characteristics curve for serum β2MG is used to differentiate between SLE patients and control group with the best cutoff point is >4.89 mg/l yielding 95% sensitivity, 97.5% specificity and 95% accuracy. In our study the mean serum Cystatin C level was statistically significantly higher in SLE patients compared to controls (P <0.001). **Gheita *et al.*** [23] performed a study on 61 SLE patients and 52 controls. They demonstrated significant increase of serum Cystatin C level in the adult SLE patients in comparison to control persons (P =0.000).

The current study demonstrated that there was a significant positive correlation between serum Cystatin C and SLEDAI-2K (P =0.049). Similarly, to results in the present study, **Lertnawapan *et al.*** [20] studied 118 patients with SLE and 83 control. They have revealed that Cystatin C was significantly correlated with disease activity in SLE. **Garcia-Garcia *et al.*** [22] also showed different results as they revealed that in SLE patients with high Cystatin C levels presented no differences in disease activity assessed by SLEDAI compared to the normal Cystatin C group.

In the current study the Receiver Operating Characteristics curve for serum Cystatin (mg/L) is used to differentiate between active and non-active with the best detected cut off point is 1.13 yielding sensitivity of 73.9%, specificity of 35.3% and total accuracy 57.5%.

Tony *et al.* [(1)] revealed that serum Cystatin C showed low sensitivity 65.0% and high specificity 100% for assessing disease activity in SLE patients. In addition, **Fatemi *et al.* [(24)]** demonstrated that Cystatin C has sensitivity to predict lupus flare and lupus activity (AUC =0.701, 95% CI =0.579-0.823, P =0.003).

In this current study the Receiver Operating Characteristics curve for Serum Cystatin C (mg/L) was used to differentiate between SLE patients and control group with the best cutoff point is >0.994 mg/l yielding 87.5% sensitivity, 62.5% specificity and 72.5% accuracy.

In agreement with the present study, **Xu *et al.* [(11)]** analyzed diagnostic efficiency of Cystatin C with ROC curve. They found that AUC of Cystatin C is excellent (AUC =0.906) for distinguishing SLE patients from healthy subjects with sensitivity75.7% specificity 94.6%.

In the present study, there was a statistically significant positive correlation between serum β2MG and Cystatin C (r =0.904, P <0.001). To the best of our knowledge, there was no other studies tested the correlation between serum β2MG and Cystatin C.

CONCLUSION

Serum β2MG and Cystatin C levels are significantly elevated in SLE patients compared to healthy subjects. Serum β2MG and Cystatin C seem to be a useful addition to laboratory tests that can help in assessment of disease activity in SLE patients. Serum β2MG and Cystatin C can serve as a good marker of renal function in SLE patients. The sensitivity, specificity and accuracy of β2MG were higher than that of Cystatin C in identifying SLE activity and differentiation between SLE patients and healthy subjects.

LIMITATIONS

Despite the promising outcomes of the current study, small sample size remains the main limitation. In addition, as administrated medications could modulate disease activity or the course of disease, the findings might be different if incident patients were enrolled. The data obtained in this study were collected at single institution and no follow up data were available. Kidney dysfunction in the current study was determined by laboratory parameters rather than by renal biopsies.

Conflict of interest: The investigators declare no conflict of interest.

Sources of funding: The current study didn't receive any specialized grant from funding agencies.

REFERENCES

1. **Tony E, Mohammed H, Fathi N *et al.* (2016)**: Serum and Urinary Biomarkers Endothelin-1, Beta-2 Microglobulin, Cystatin C, Galectin-3 and Alpha-1-acid Glycoprotein; Can they Surrogate Clinical and Histological Staging in Lupus Nephritis Patients? Journal of Arthritis, 5(6):1-9.
2. **Żychowska I, Suszek D, Dryglewska M *et al.* (2018)**: β2-microglobulin as a marker of systemic lupus erythematosus activity. Advances in Clinical and Experimental Medicine: Official Organ Wroclaw Medical University, 27(3):379-82.
3. **Badr A, El-Melligy D, Mostafa H *et al.* (2005)**: 1 Evaluation of Beta2-Microglobulin as a Possible Biomarker for Assessment of Disease Activity in Systemic Lupus Erythematosus and Chronic Kidney Disease. World J Med Sci., 12(1):26-35.
4. **McPhee C, Sproule T, Shin D *et al.* (2011)**: MHC class I family proteins retard systemic lupus erythematosus autoimmunity and B cell lymphomagenesis. The Journal of Immunology, 187(9):4695-704.
5. **Kim H, Jeon J, Yoon J *et al.* (2010)**: Beta2-microglobulin can be a disease activity marker in systemic lupus erythematosus. The American Journal of the Medical Sciences, 339(4):337-40.
6. **Skare T, Ferri K, Santos M (2014)**: Systemic lupus erythematosus activity and beta two microglobulin levels. Sao Paulo Medical Journal, 132:239-42.
7. **Liu B, Lu F, Lin J *et al.* (2021)**: Correlations of serum beta 2-microglobulin level with disease activity and renal involvement in patients with systemic lupus erythematosus. Journal of Laboratory Medicine, 45(1):13-8.
8. **Chew C, Pemberton P, Husain A *et al.* (2013)**: Serum cystatin C is independently associated with renal impairment and high sensitivity C-reactive protein in systemic lupus erythematosus. Clin Exp Rheumatol., 31(2):251-5.
9. **Nishiyama S, Ohashi K, Aita T *et al.* (2014)**: AB0551 Cystatin C Associates with Disease Activity in Patients with Systemic Lupus Erythematosus Independent of Renal Function. Annals of the Rheumatic Diseases, 73(2):988. http://dx.doi.org/10.1136/annrheumdis-2014-eular.5003
10. **Waldman M, Appel G (2006)**: Update on the treatment of lupus nephritis. Kidney International, 70(8):1403-12.
11. **Xu B, Zhang Y, Yang Y *et al.* (2019)**: Diagnostic performance of serum cystatin C and complement component 1q in lupus nephritis. Arthritis Research & Therapy, 21(1):1-10.
12. **Aringer M, Costenbader K, Daikh D *et al.* (2019)**: 2019 European League Against Rheumatism/American College of Rheumatology classification criteria for systemic lupus erythematosus. Arthritis & Rheumatology, 71(9):1400-12.
13. **Gladman D, Ibanez D, Urowitz M (2002)**: Systemic lupus erythematosus disease activity index 2000. The Journal of Rheumatology, 29(2):288-91.
14. **Levey A, Coresh J, Balk E *et al.* (2003)**: National Kidney Foundation practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Annals of Internal Medicine, 139(2):137-47.

15. **Polachek A, Gladman D, Su J *et al.*** (**2017**): Defining low disease activity in systemic lupus erythematosus. Arthritis Care & Research, 69(7):997-1003.
16. **Abd-Elbaky N, Albeltagy E, Hammour A *et al.*** (**2019**): Evaluation of serum β2-microglobulin in Egyptian patients with systemic lupus erythematosus. Egypt J Immunol., 26:143-53.
17. **Silva M, Moscoso-Solorzano G, Nishida S *et al.*** (**2012**): Serum Beta 2-microglobulin/cystatin C index: a useful biomarker in lupus nephritis. Nephron Extra., 2(1):169-76.
18. **Aghdashi M, Salami S, Nezhadisalami A** (**2019**): Evaluation of the serum β2 Microglobulin level in patients with systemic lupus erythematosus and its correlation with disease activity. BioMedicine, 9(3):16. doi: 10.1051/bmdcn/2019090316
19. **Wakabayashi K, Inokuma S, Matsubara E *et al.*** (**2013**): Serum β2-microglobulin level is a useful indicator of disease activity and hemophagocytic syndrome complication in systemic lupus erythematosus and adult-onset Still's disease. Clinical Rheumatology, 32(7):999-1005.
20. **Lertnawapan R, Bian A, Rho Y *et al.*** (**2012**): Cystatin C is associated with inflammation but not atherosclerosis in systemic lupus erythematosus. Lupus, 21(3):279-87.
21. **El-Shafey A, Hammad M, Sharaf D *et al.*** (**2015**): Evaluation of Serum Cystatin C as a Marker of Glomerular filtration rate in Patients with Systemic Lupus Erythematosus. International Journal, 3(5):372-80.
22. **Garcia-Garcia P, Castejon R, Tutor-Ureta P *et al.*** (**2017**): Serum cystatin C is associated with kidney function but not with cardiovascular risk factors or subclinical atherosclerosis in patients with Systemic Lupus Erythematosus. Clinical Rheumatology, 36(12):2709-17.
23. **Gheita T, Abd El Baky A, Baky E *et al.*** (**2015**): Serum cystatin C, urinary neutrophil gelatinase-associated lipocalin and N-acetyl-beta-D-glucosaminidase in juvenile and adult patients with systemic lupus erythematosus: Correlation with clinical manifestations, disease activity and damage. Saudi Journal of Kidney Diseases and Transplantation, 26(3):497-506.
24. **Fatemi A, Rahami Z, Smiley A** (**2021**): Role of cystatin C in predicting disease activity and flare-up in systemic lupus erythematosus: A longitudinal follow-up study. International Journal of Preventive Medicine, 12:63. doi: 10.4103/ijpvm.IJPVM_619_20.