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ORIGINAL ARTICLE

Serum cystatin C and βeta-2 microglobulin as potential biomarkers in children with lupus nephritis

Eman Baraka[1], Nashwa Hashaad[1], Walid Abdelhalim[2], Gehan Elolemy[1]

[1]*Department of Physical Medicine and Rehabilitation, Division of Rheumatology, Faculty of Medicine, Benha University, Benha, Egypt*
[2]*Department of Clinical and Chemical Pathology, Faculty of Medicine, Benha University, Benha, Egypt*

ABSTRACT

Objectives: In this study, we aimed to assess serum levels of Cystatin C (Cys C) and beta-2 microglobulin (β2M) in juvenile systemic lupus erythematosus (JSLE) patients and to investigate their role as potential biomarkers of lupus nephritis (LN) and overall disease activity.

Patients and methods: Between December 2018 and November 2019, a total of 40 patients with JSLE (11 males, 29 females; mean age: 12.6±2.5 years; range, 7.5 to 16 years) and 40 age- and sex-matched controls (10 males, 30 females; mean age: 12.3±2.4 years; range, 7 to 16 years) were included in this study. Serum (s) Cys C and β2M levels were compared between the groups. The SLE Disease Activity Index (SLEDAI-2K), the renal SLEDAI (rSLEDAI), and the Renal Damage Index were used.

Results: JSLE patients had significantly elevated mean sCyc C and sβ2M levels (1.4±0.8 mg/mL and 2.8±0.9 mg/mL, respectively) compared to the controls (0.6±0.1 mg/mL and 2.0±0.2 mg/mL, respectively; p<0.00). The mean sCys C and sβ2M levels were significantly higher in the LN group, compared to non-LN patients (1.8±0.7 mg/mL and 3.1±1.0 mg/mL, respectively vs. 0.8±0.3 mg/mL and 2.4±0.6 mg/mL, respectively; p=0.002 and p=0.02, respectively). The sCys C levels had significant positive correlations with erythrocyte sedimentation rate (r=0.3, p=0.05), serum creatinine (r=0.41, p= 0.007), 24-h urinary protein (r=0.58, p<0.001), anti-double stranded deoxyribonucleic acid antibodies titers (r=0.55, p=0.002), extra-renal SLEDAI scores (r=0.36, p=0.04), rSLEDAI (r=0.46, p=0.002), and renal class (r=0.7, p=0.0001). Serum β2M levels were significantly negatively correlated with complement 4 levels (r=-0.31, p=0.04) and significantly positively correlated with extra-renal SLEDAI scores (r=0.3, p=0.05).

Conclusion: These findings confirm that sCys C and sβ2M levels are increased in JSLE patients in association with the overall active disease. However, sCys C level may act as a promising non-invasive biomarker for predicting kidney disease activity and biopsy classes in children with JSLE.

Keywords: Cystatin C, disease activity, juvenile lupus nephritis, renal biopsy, βeta-2 microglobulin.

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by multisystem immune-mediated tissue damage and heterogeneous clinical manifestations.[1] The disease begins in 10 to 20% of patients prior to adulthood and is called juvenile SLE (JSLE), as 16 years of age is the most commonly used maximum age in the diagnosis of JSLE, while 14 to 20 years of age have been adopted.[2] Juvenile SLE affects females more commonly, probably due to hormonal changes in puberty, yet the ratio of girls and boys (5 to 8:1) is less noticeable compared to adults, which increases with age to adult values.[3] Although the clinical features of JSLE are similar to those observed in adult SLE patients, the disease is characterized by a higher activity and frequent renal, hematological, and neuropsychiatric involvement in children.[4]

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Correspondence: Eman Baraka, MD. Department of Physical Medicine and Rehabilitation, Division of Rheumatology, Faculty of Medicine, Benha University, 15318 Benha, Egypt. E-mail: dremanbaraka@yahoo.com

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Lupus nephritis (LN) affects up to 80% of JSLE patients and develops in more than 90% of patients within two years of diagnosis with the potential for irreversible kidney damage in up to 19% of patients. Since the late diagnosis of renal impairment is associated with a high incidence of end-stage renal disease (ESRD), early diagnosis and prompt intervention are of great importance.[5,6]

Renal biopsy plays a crucial role in the diagnosis of LN and the pathological classification by the International Society of Nephrology and the Renal Pathology Society (ISN/RPS) or the earlier classifications by the World Health Organization (WHO) and scored activity indices (AIs) and chronicity indices (CIs) are also used to predict the renal prognosis and for therapy guidance.[7,8]

However, renal biopsy carries a risk of bleeding complications.[9] Besides, serial biopsies may be required to inform ongoing treatment decisions and predict long-term prognosis, which makes it difficult to apply, particularly in JSLE patients.[10] Several biomarkers have been actively sought to replace invasive kidney biopsies such as proteinuria, creatinine (Cr) clearance, anti-double stranded deoxyribonucleic acid antibodies titers (anti-dsDNA) levels and serum complement, and have found that they may not be useful to adequately predict relapse or identify the degree of disease activity and chronic damage.[11]

Cystatin C (Cys C) is a small-molecular-weight endogenous protein which belongs to the cysteine protease inhibitors, and its serum level has been suggested as a sensitive and stable marker of glomerular filtration rate (GFR). It is produced by all nucleated cells at a relatively constant rate, as a housekeeping gene encodes the protein.[12] Under normal circumstances, the serum level of Cys C is almost completely filtered by the renal glomeruli and catabolized primarily by the proximal tubules; thus, its levels are closely correlated with GFR.[13]

Beta-2 microglobulin (β2M) is a low-molecular-weight protein that is primarily released by active lymphocytes, and increased serum levels have been detected in patients with rheumatoid arthritis, Sjögren's syndrome, and SLE.[14] The circulating β2M is reabsorbed by proximal renal tubule and its enhanced urinary excretion is a known marker of tubulointerstitial renal diseases.[15] The molecule was considered a relatively non-toxic uremic retention solute; however, its significance as a potential non-Cr renal filtration marker was overshadowed by Cys.[16]

There are constant searches to meet the unmet needs of sensitive biomarkers for chronic kidney disease. In the present study, therefore, we aimed to assess serum levels of Cys C and β2M in JSLE patients and to investigate their role as potential biomarkers of LN and overall disease activity.

PATIENTS AND METHODS

This cross-sectional study was conducted at the Rheumatology, Rehabilitation and Physical Medicine Departement. Faculty of Medicine, Benha University between December 2018 and November 2019. A total of 40 patients with JSLE (11 males, 29 females; mean age: 12.6±2.5 years; range, 7.5 to 16 years) who met at least four of the revised American College of Rheumatology criteria for the classification of SLE[17] were included. All patients were recruited from the inpatient section and outpatient clinic of the hospital. Exclusion criteria included any history suggestive of any systemic, autoimmune, or connective tissue diseases. In addition, those with overlap autoimmune diseases, active infections (such as viral hepatitis and tuberculosis), ESRD, kidney transplantation, malignancy, or those using non-steroidal anti-inflammatory drugs (NSAIDs) during the past six months were excluded. The control group consisted of 40 age- and sex-matched healthy volunteers (10 males, 30 females; mean age: 12.3±2.4 years; range, 7 to 16 years). Children of the control group were recruited from the children of hospital workers and their relatives. Inclusion criteria for the control group were as follows: age <16 years and absence of history suggesting of any systemic, autoimmune, or connective tissue diseases. In addition, their laboratory assays including inflammatory markers, renal function test and immunology work-up were within the normal ranges. All clinical assessments and laboratory investigations were done on the same day of sample collection.

All demographic data, full history, and current medications were recorded. Participants' height and weight were estimated,

body mass index (BMI) was calculated, and a thorough clinical examination was performed. The Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) was used to assess disease activity.[18] For the assessment of renal disease activity, the renal SLEDAI (rSLEDAI) was used. It represents the sum of the four renal-related items of the SLEDAI-2K; hematuria and pyuria (both >5 cells/high power field), proteinuria >0.5 g/day, and cellular casts. Each of the four items receives a score of 4, and the total rSLEDAI score ranges from 0 (inactive renal disease) to a maximum of 16. Extra-renal disease activity was assessed using the extra-renal SLEDAI, which includes the sum of all other domains' scores excluding those of four renal domains in the SLEDAI-2K score.

Cumulative renal damage was assessed using the renal-related items of the Systemic Lupus International Collaborating Clinics/American College of Rheumatology damage index (rSLICC).[19] Items which specifically characterize kidney damage include estimated GFR <50%, proteinuria ≥3.5 gm/24 h, and ESRD. However, the presence of ESRD was one of the exclusion criteria in our study and, therefore, rSLICC ranged from 1 to 2. The results of renal biopsy that was done as a part of disease evaluation of the JSLE (up to three months prior to study entry) were considered and classified according to the WHO classification system for LN.[7] Activity (AIs) and chronicity (CIs) indices for biopsy assessment of LN were recorded.[8]

A total of 7 mL of venous blood sample were taken from the participants in this study and used for laboratory investigations. The blood sample was divided into three parts: the first part was used freshly for measurement of complete blood count (CBC) by Sysmex XP-300 (Sysmex Corporation, Kobe, Japan), and the second part was used for measurement of the erythrocyte sedimentation rate (ESR 1st h), and C-reactive protein (CRP) mg/mL, and the third part was allowed to clot, then centrifuged (2,000 to 3,000 rpm), and the separated serum was used to measure serum complement (C3 and C4), anti-nuclear antibody (ANA), and anti-dsDNA. Renal function was assessed by serum Cr, blood urea nitrogen (BUN), urine analysis, and 24-h urine protein. The GFR was estimated (eGFR) in (mL/min/1.73 m^2)

Table 1. Characteristics of the study participants

	JSLE group (n=40)		Control group (n=40)		
	n	Mean±SD	n	Mean±SD	*p*
Age (year)		12.6±2.5		12.3±2.4	0.61
Sex					0.79
Male	11		10		
Female	29		30		
Body mass index (kg/m^2)		15.2±4.7		14.3±4.0	0.36
Erythrocyte sedimentation rate (mm/1st h)		71.5±14.9		14.1±6.0	<0.001*
C- reactive protein (mg/mL)		8.8±6.0		3.5±1.6	<0.001*
Serum creatinine (mg/DL)		1.0±0.0		0.8±0.6	0.06
Blood urea nitrogen (mg/dL)		155.9±5.5		13.9±3.9	0.06
Protein/creatinine ratio		1.3±1.3		0.2±0.1	<0.001*
Estimated glomerular filtration rate (mL/min/1.73 m^2)		78.0±28.9		93.2±7.8	0.2
Anti-dsDNA Abs (U/mL)		48.9±36.2		NA	-
C3 (mg/dL)		111.1±27.6		NA	-
C4 (mg/dL)		2.0±9.8		NA	-
Serum Cys C mg/L		1.4±0.8		0.6±0.1	<0.001*
Serum β2M mg/L		2.8±0.9		2.0±0.2	<0.001*

JSLE: Juvenile systemic lupus erythematosus; SD: Standard deviation; ds-DNA Abs: Double stranded DNA antibodies; C3: Complement 3; C4: Complement 4; Cys C: Cystatin C; β2M: Beta-2 microglobulin; P<0.05: Insignificant; P<0.05*: Significant.

according to the Bedside Schwartz formula [41.3 × (height in meters/serum Cr in mg/dL)].[20] The remaining serum was kept frozen at -20 for the measurement of serum Cys C (Cat#: MBS700210, MyBioSource, San Diego, CA, USA), serum β2M (Cat#: MBS705051, MyBioSource, San Diego, CA, USA) using enzyme-linked immune sorbent assay (ELISA) in accordance with the manufacturer instructions.

Statistical analysis

Statistical analysis was performed using the SPSS version 15.0 software (SPSS Inc., Chicago, IL, USA). Data were presented in mean ± standard deviation (SD), median (interquartile range [IQR]) or number and percentage. The Student's t-test, Mann-Whitney U test, or chi-square test were to compare variables between the groups, where applicable. The Spearman correlation was used to identify any correlation between two variables. A multiple regression analysis was performed to examine independent variables which could predict renal biopsy class. A *p* value of <0.05 was considered statistically significant.

RESULTS

Forty children with JSLE and 40 apparently healthy children were recruited over a 12-month period. Age, sex, and BMI distributions were matched in both groups (p=0.61, p=0.79, and p=0.36, respectively). Children with JSLE had an elevated mean serum Cyc C and serum β2M levels (1.4±0.8 mg/mL and 2.8±0.9 mg/mL, respectively), compared to healthy controls (0.6±0.1 mg/mL and 2.0±0.2 mg/mL, respectively; p<0.001) (Table 1).

The mean disease duration was 17.5+10.2 (range, 3 to 42) months. The most common clinical manifestations of the patients at the time of the study were arthralgia and/or arthritis (82.5%), LN (57.5%), hematological disorders

Table 2. Clinical characteristics of the children with juvenile systemic lupus erythematosus

	JSLE group (n=40)				
Parameter	n	%	Mean±SD	Median	IQR
Disease duration (month)			17.5±10.2		
Clinical manifestations.					
Arthralgia and/arthritis	33	82.5			
Malar rash	18	45			
Photosensitivity	12	30			
Oral ulcers	9	22.5			
Alopecia	15	37.5			
Discoid rash	8	20			
Serositis	15	37.5			
Hematological	26	56			
Renal	23	57.5			
Neurologic	9	22.5			
SLEDAI			12.9±9.1		
Current medications					
Corticosteroids	24	60			
HCQ	36	90			
Azathioprine	19	47.5			
MMF	8	20			
Intravenous cyclophosphamide	1	2.5			
Renal histopathology					
Class					
II	7	30.4			
III	4	17.4			
IV	4	17.4			
Activity index				11	8-14.5
Chronicity index				3	2-4

JSLE: Juvenile systemic lupus erythematosus; SD: Standard deviation; IQR: Interquartile range; SLEDAI: Systemic lupus erythematosus disease activity index; HCQ: Hydroxychloroquine; MMF: Mychophenolate mofetil.

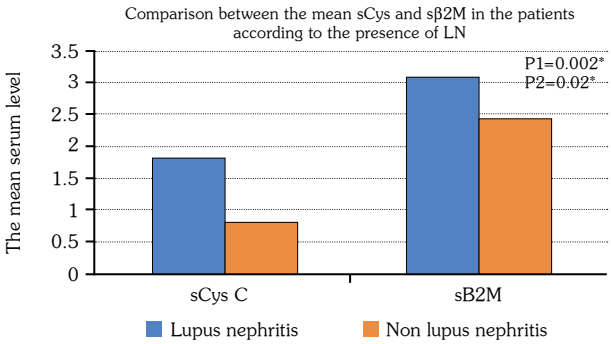

Figure 1. Showed comparison between the mean serum Cystatin C (Cys C) and β2M levels according to the presence of lupus nephritis. Levels of serum Cys C and β2M were significantly higher in the LN group compared to non-LN patients (1.8±0.7 mg/mL and 3.1±1.0 mg/mL respectively) *vs.* (0.8±0.3 mg/mL and 2.4±0.6 mg/mL respectively).

P<0.05*: Significant; LN: Lupus nephritis.

(56%), and malar rash (45%) (Table 2). Fifteen (37.5%) patients previously underwent a renal biopsy as a part of disease evaluation and were classified according to the WHO classification system for LN. The patients' medication at the time of blood withdrawal, clinical manifestations, as well as histopathological features of renal biopsies (classes, AIs and CIs) are shown in Table 2.

The patients were further divided into two groups as the LN group (n=23), including patients with nephritis (clinically and/or biopsy-proved) and as the non-LN group (n=17) including those without nephritis. Musculoskeletal and mucocutaneous manifestations were the most common clinical features in both groups, followed by hematological disorders. Patients with LN had a significantly higher titer of anti-dsDNA (p<0.001) and proteinuria (p<0.001) and significantly lower levels of C3 (p=0.005), compared with non-LN patients. The mean values of ESR (p=0.06), CRP (p=0.93), serum Cr (p=0.06), BUN (p=0.61), and eGFR (p=0.14) did not show any statistically significant difference between the patients with

Table 3. Comparison of the demographic, clinical and laboratory characteristics between the JSLE patients with and without nephritis

	LN group (n=23)				Non-LN group (n=17)				
Variable	n	Mean±SD	Median	IQR	n	Mean±SD	Median	IQR	*p*
Age (year)		12.2±2.7				13.2±2.1			0.24
Sex									0.23
Male	8				3				
Female	15				14				
Body mass index (kg/m^2)		15.4±5.3				14.1±3.4			0.23
Disease duration (month)		17.7±11.1				17.2±8.8			0.87
Erythrocyte sedimentation rate (mm/1st h)		75.4±14.6				66.2±13.7			0.06
C- reactive protein (mg/mL)		8.7±5.9				8.7±5.9			0.93
Serum Cr (mg/DL)		1.0±0.7				0.7±0.1			0.06
Blood urea nitrogen (mg/dL)		16.5±5.5				15.6±5.4			0.61
Protein in 24h urine (gm/24h)		2.1±1.2				0.2±0.1			<0.001*
eGFR (ml/min/1.73 m^2)		81.2±35.7				93.9±11.5			0.14
Anti-dsDNA Abs (U/mL)		70.0±34.6				20.5±8.0			<0.001*
C3 (mg/dL)		100.9±29.4				124.8±6.9			0.005*
C4 (mg/dL)		18.0±9.8				22.6±9.2			0.06
Extra-renal SLEDAI		8.3±6.0				6.5±5.7			0.36
rSLEDAI		9.2±3.4				-			-
rSLICC			1	0-1			-	-	-
Serum CysC mg/L		1.8±0.7				0.8±0.3			0.002*
Serum β2M mg/L		3.1±1.0				2.4±0.6			0.02*

JSLE: Juvenile systemic lupus erythematosus; LN: Lupus nephritis; SD: Standard deviation; IQR: Interquartile range; h: hour; Cr: Creatinine; eGFR: Estimated glomerular filtration rate; dsDNA Abs: Double stranded DNA antibodies; C3: Complement 3; C4: Complement 4; SLEDAI: Systemic lupus erythematosus disease activity index; r: Renal; SLICC: Systemic Lupus International Collaborating Clinics/American College of Rheumatology damage index; Cys C: Cystatin C; β2M: Beta-2 microglobulin; P<0.05: Insignificant; P<0.05*: Significant.

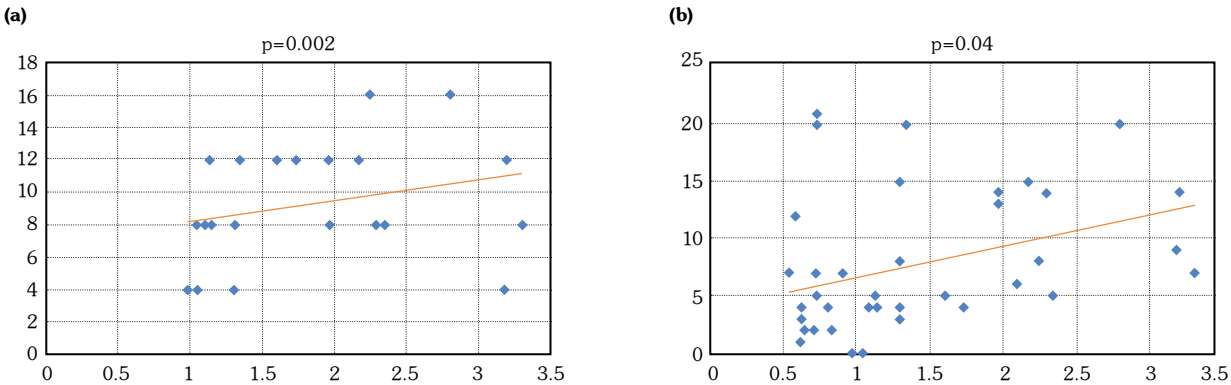

Figure 2. (a) Showed that cystatin C serum levels were significantly positively correlated with the values of extra-renal SLEDAI scores (r=0.36, p=0.04*) and **(b)** the values of rSLEDAI scores (r=0.46, p=0.002).

SLEDAI: Systemic lupus erythematosus disease activity index; rSLEDAI: Renal systemic lupus erythematosus disease activity index; p<0.05*: Significant.

and without LN. Also, the mean extra-renal SLEDAI scores did not differ significantly between the LN and non-LN groups (8.3±6.0 *vs.* 6.5±5.7, respectively; p=0.31). However, the mean levels of serum Cys C and β2M were significantly higher in the LN group, compared to non-LN patients (1.8±0.7 mg/mL and 3.1±1.0 mg/mL, respectively *vs.* (0.8±0.3 mg/mL and 2.4±0.6 mg/mL,

Table 4. Correlation between serum Cys C and serum β2M levels with the clinical, laboratory and pathological characteristics of the patients

	Serum β2M		Serum Cys C	
	r	*p*	r	*p*
Age	0.17	0.30	0.09	0.55
Disease duration	0.16	0.31	0.02	0.87
Body mass index	0.08	0.60	0.03	0.83
Erythrocyte sedimentation rate (mm/1st h)	0.22	0.16	0.3	0.05*
C- reactive protein (mg/mL)	0.26	0.26	0.13	0.39
Serum Cr (mg/DL)	0.33	0.83	0.41	0.007*
Blood urea nitrogen (mg/dL)	0.07	0.62	0.6	0.32
Protein in 24 h urine (gm/24 h)	0.25	0.11	0.58	0.001*
Estimated glomerular filtration rate (mL/min/1.73 m²)	-0.09	0.55	-0.35	0.02*
Anti-dsDNA Abs (U/mL)	0.07	0.65	0.55	0.002*
C3 (mg/dL)	- 0.27	0.08	- 0.47	0.002*
C4 (mg/dL)	- 0.31	0.04*	- 0.26	0.09
Extra-renal SLEDAI	0.03	0.05*	0.36	0.04*
rSLEDA	0.16	0.16	0.46	0.002*
rSLICC	0.94	0.94	0.19	0.38
Renal class	0.88	0.88	0.7	0.0001*
Activity index	0.02	0.89	0.14	0.5
Chronicity index	0.09	0.67	0.16	0.22
Serum Cys C	0.21	0.19	1	1
Serum β2M	1	-	0.21	0.19

Cr: Creatinine; dsDNA Abs: Double stranded DNA antibodies; C3: Complement 3; C4: Complement 4; SLEDAI: Systemic lupus erythematosus disease activity index; r: Renal; SLICC: Systemic Lupus International Collaborating Clinics/American College of Rheumatology damage index; Cys C: Cystatin C; β2M: Beta-2 microglobulin; p<0.05: Insignificant; p≤0.05*: Significant.

respectively; p=0.002 and p=0.02, respectively) (Figure 1). Comparison of the demographic, clinical, and laboratory characteristics between both groups is shown in Table 3.

The serum Cys C levels were significantly positively correlated with the values of ESR (r=0.3, p=0.05), serum Cr (r=0.41, p=0.007), 24-h urinary protein (r=0.58, p<0.001), anti-dsDNA titers (r=0.55, p=0.002), and both extra-renal SLEDAI scores (r=0.36, p=0.04 (Figure 2a) and rSLEDAI (r=0.46, p=0.002 (Figure 2b), as well as histopathological renal class (r=0.7, p=0.0001). However, these levels were significantly negatively correlated with eGFR (r=-0.35, p=0.02) and C3 levels (r=-0.47, p=0.002). On the other hand, the serum β2M levels were significantly negatively correlated with C4 levels (r=-0.31, p=0.04) and significantly positively correlated with extra-renal SLEDAI scores (r=0.3, p=0.05). In contrast, neither serum Cys C nor β2M levels showed a significant association with the rSLICC (p=0.38 and p=0.94, respectively), AIs (p=0.5 and p=0.89, respectively) and CIs (p=0.22 and p=0.67, respectively). Serum Cys C and β2M levels were not correlated with each other (r=0.21, p=0.19, respectively) (Table 4).

Among other independent variables, including 24-h urinary protein, serum Cr, eGFR, anti-dsDNA and serum β2M, the level of serum Cys C was identified as the most significant predictor of the renal histopathological class (r=0.93, p=0.04).

DISCUSSION

Although the same criteria are applied to classify SLE regardless of the age of patients, there are important differences between JSLE and adult-onset SLE. Remission is uncommon in adult patients with SLE and extremely rare with JSLE.[21] Moreover, a higher frequency of renal involvement has been demonstrated in children with SLE, compared to those with adult-onset disease with an increased need for high steroid doses and immunosuppressive therapies showing showed various toxicities and exerting tremendous effects in vulnerable phases of child's growth and development.[22] These data highlight the heavy burden of renal disease in children with SLE and the importance of early diagnosis and prompt treatment for better outcomes.[23]

Microscopic hematuria, nephrotic syndrome, hypertension, and acute renal injury are common presentations of LN in children.[24] However, some patients may be asymptomatic despite having significant renal damage, based on conventional markers of the disease.[25,26] Also, LN develops in about 50 to 70% of adults and 37 to 82% of children with SLE.[24] Consistent with previous reports, LN was demonstrated in 57.5% of the children with SLE as one of the most prevalent manifestations in our study.

In the current study, there was no statistically significant difference in the mean GFR between patients and healthy children and they were also comparable between patients with and without LN. The glomerular defect is usually more severe and clinically significant than the tubular defect in LN and GFR should be determined with an appropriate and affordable method.[27]

Although affected by age, muscle mass, and GFR, serum Cr remains the most frequently used test for detecting glomerular filtration defects.[28] The decrease in GFR is reflected by an increase in serum Cr, but it was interesting that serum Cr and BUN were found within normal values in both patients and controls in our study with a trend toward higher values in patients, particularly those with LN; however, the results were still comparable. The use of serum Cr as a screening test is limited by the fact that it may remain within normal values of the population-based reference interval in patients with abnormal GFR due to a large inter-individual variation and relatively small intra-individual variation.[29] Furthermore, serum Cr levels may change separately from the glomerular function with eating meat, malnutrition, muscle atrophy, liver disease, or increased tubular secretion.[30]

The dearth of biomarkers in childhood hampers the proper management and development of novel therapies, particularly in childhood, as the treatment of severe LN is usually associated with unsatisfactory outcomes.[31] Significant efforts have been made in identifying biomarkers that can predict impending renal flare, follow renal disease progression, and monitor treatment response.[32]

Cys C and β2M are low-molecular-weight proteins that have been investigated as potential markers to accurately assess renal function.[33] Due to its low molecular weight and a positive charge at physiological pH levels, Cys C is filtered almost freely by the glomeruli along with a constant production rate and, thus, the level of Cys C is mainly determined by GFR.[13] Although published studies have shown controversial results,[34-36] serum β2M was proposed to serve as an accurate renal biomarker as being not influenced by age, sex, or muscle mass.[33] Also, β2M urinary excretion has been used in the diagnosis of a variety of kidney diseases in children.[16] As shown in the present study, children suffering from LN had significantly higher proteinuria and anti-dsDNA titers, and lower C3 levels, consistent with the reported data.[37]

Although anti-dsDNA and complement components are among the various parameters routinely used to assess SLE activity in daily practice, their utility remains controversial.[38] The complement activation is not always accurately reflected by changes in C3 and C4 levels,[38,39] and C3 can act as an acute-phase protein; therefore, its consumption may be masked by increased production.[40] Low-grade albuminuria is usually considered an early marker of kidney disease; however, there is an emerging view that it can be also a sign of generalized vascular disease, such as endothelial dysfunction.[41]

In the current study, the mean serum Cys C and β2M levels were significantly higher in patients with JSLE, compared to healthy children and in patients with LN than in those without LN. Studies of Cys C and β2M in JSLE are scarce; however, our results are consistent with the results of an Egyptian study which reported that serum Cys C was significantly higher in both adults and children with SLE, compared to healthy individuals, adding that its level was also higher in SLE patients with kidney injury.[42] Similar results have been demonstrated in adult patients with SLE, where an increase in serum Cys C has been reported in patients with a history of nephritis even after adjusting conventional measures of renal function.[36,37,43,44]

We found that serum Cys C was correlated with conventional markers of renal impairment, as well as indices of disease activity. In agreement with our results, a strong association between serum Cys C and conventional indicators of renal impairment including 24-h urine protein and eGFR in addition to SLE activity (SLEDAI) in patients with LN was reported in a previous study.[45] In the same study, serum Cys C also showed a better statistical p value in response to corticosteroid and cyclophosphamide treatment than serum Cr and BUN.[45] Another study supported these findings by demonstrating that serum Cys C correlated positively with serum Cr and negatively with Cr clearance and eGFR.[44] A study including 226 patients with different renal pathologies showing glomerular and tubular impairments showed that serum Cys C was superior to serum Cr to detect reduced GFR independently of either glomerular or tubular dysfunction.[46] Similarly, serum Cys C was proved to be a reliable marker of GFR in patients with decreased GFR.[47] and was found to be even more effective than serum Cr detecting reduced GFR in patients suffering from type 2 diabetes mellitus.[48] As demonstrated in our results, serum Cys C was identified as the most significant predictor of the renal histopathological class, but not of AIs or CIs. While some authors reported a significant association between Cys C and both the damage index and kidney biopsy class,[42] others were unable to find any role for Cys C in predicting biopsy classes; however, they did not find a correlation between serum Cys C levels and markers of activity or chronicity, either.[37] To the best of our knowledge, we are the first to study the relationship of serum β2M in children with LN. Our results supported the previously reported findings, confirming a significant increase in serum β2M in SLE patients.[37,49-51]

Although there was a significant increase in the serum β2M in JSLE patients with LN, its levels were not associated with renal laboratory or histopathological parameters. However, we found significant correlations between serum levels of β2M and extra-rSLEDAI, but not rSLEDAI. Several studies have shown a significant association between serum β2M levels and activity parameters in SLE patients, including SLEDAI and it is assumed to be a good marker for detecting SLE activity.[15,52,53]

Conversely, an insignificant difference in serum β2M between the low and high SLEDAI groups was reported in another study, although the authors found a significant

difference in urinary β2M between the same groups. Increased serum levels of β2M may be a result of lymphocyte turnover during lymphoproliferative and autoimmune diseases[16] or the presence of immune complexes that are eliminated by the kidneys.[53] In addition, an insignificant correlation between serum and urinary β2M with activity and chronicity indices were identified by others and claimed that this marker could not be used to assess or monitor LN activity.[37]

Our study has limitations. Since it is an invasive procedure, not all JSLE children included in the study had a renal biopsy performed. However, our results demonstrated a significant link between serum Cys C levels and renal biopsy classes. As a cross-sectional study, we had no opportunity to evaluate the relationship with glucocorticoids and immunosuppressive agents and to assess their effect on serum levels of Cys C and β2M. Therefore, a larger multi-center study with a greater number of renal biopsy and prospective design is highly recommended to further investigate the diagnostic values and predictive properties of Cys C in children with LN.

In conclusion, these findings confirm that serum Cys C and β2M levels are increased in JSLE patients in association with the overall active disease. However, serum Cys C level may act as a promising non-invasive biomarker for predicting kidney disease activity and biopsy classes in children with JSLE.

Ethics Committee Approval: The study protocol was approved by the Faculty of Medicine, Benha University Research Ethics Committee (date: 14.10.2018, no: 000066). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Patient Consent for Publication: A written informed consent was obtained from each parent and/or legal guardian of the patient.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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