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Value of soluble CD163/ as a biomarker for diagnosis/ and evaluation of lupus nephritis

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Abstract:

Introduction: Systemic lupus erythematosus (SLE) is a multisystemic illness characterized by a remitting and relapsing nature. The renal system is among the most important and prevalent affected systems in Systemic lupus and may result in morbidity and death. Urinary markers, such as Urine Soluble Cluster of Differentiation 163 (US CD163), have been explored as an indicator for lupus nephritis (LN)flare in cases with SLE.

Aim of the study: This research aimed to assess urine soluble CD163 levels in SLE cases as a potential marker for LN diagnosis and activity assessment.

Subjects and Methods: The study enrolled 52 cases (46 Females and 6 males) along with 25 gender and age-matched controls. A full examination, investigations, and systemic lupus disease activity index (SLEDAI) were conducted on patients. Our marker urine soluble CD163 was measured in urine samples of patients by (ELISA technique. The receiver operating characteristic (ROC) curve has been utilized to ascertain the discriminatory value of urine soluble CD163 for differentiating cases with active lupus nephritis (ALN) from cases with no renal flare.

Results: A case control study included six men and 46 women, with an average age of (33.6±8.8) y. There were 27 active LN and 25 NRA systemic lupus cases. Urine sCD163 levels were statistically significantly higher in systemic lupus cases (7±3.6) compared to controls (4.4 ±1.03, $p < 0.001$). ALN cases showed considerably greater levels of US CD163(9.3 ±3.5) with p<0.001compared to no renal activity (NRA) SLE patients (4.5 ± 1.3) and controls ($p < 0.001$). The ideal cut-off value for normalized urine soluble CD 163 to anticipate kidney activity was > 4.58, 75% sensitivity, 52% specificity, and $p < 0.001$. The current investigation found that urine soluble CD163 levels are linked with total SLEDAI (P =0.001) and rSLEDAI (P = 0.001).

Conclusions: Us CD163 might be a measure of renal flare in systemic lupus cases. It distinguishes between SLE cases with ALN and SLE cases with NRA. Its quantity is associated with symptoms of systemic lupus and laboratory tests that show renal illness activity. Consequently, it serves as an indicator of LN activity.

Keywords: LN; urine soluble CD163; Urinary biomarker; SLE.

1. Introduction

SLE is a multisystemic illness that occurs in a remitting and relapsing course [1]. LN is a dangerous and most prevalent symptom of systemic lupus that can lead to a high death rate and morbidity [2]. Recent research has focused on urine sediments in the search for novel lupus nephritis biomarkers that potentially surmount the constraints of existing ones. They can precisely represent the immune-inflammatory environment of the kidney and its pathological association, which would provide important data in the search for novel markers. The predominant urinary cells in the active sediment of LN are macrophages [3].

Known as a scavenger receptor for hemoglobin-haptoglobin complexes, Cluster of Differentiation 163 is a transmembrane protein that weighs 130 kilodaltons and is a member of the cysteine-rich scavenger receptor superfamily type B. These receptors are produced by M2c macrophages, which are macrophages that penetrate tissue during the phase of inflammation that is dedicated to repair [4] and [5]. These macrophages to an M2c phenotype is a reaction to the environment [4, 6, 7]. CD163+ cells were found in cellular crescent lesions and tubulointerstitial lesions in cases with lupus nephritis [8, 9]. Soluble CD163 is the result of metalloproteinases that break down the CD163 macrophage receptor [10, 11]. After breakdown, soluble CD163 appears in urine and can be evaluated in active renal illness, like LN and ANCA-associated vasculitis [12, 13].

The purpose of this research is to evaluate urine soluble CD163 levels in systemic lupus cases with or without active LN at the time of the research as a potential indicator for LN diagnosis and activity assessment.

2. Subjects and methods

2.1. Subjects

Written informed consent was taken from all patients. Sample size calculated from the equation: $n = (Z\alpha/2 + Z\beta)^2 * [p_0(1-p_0) + p_1(1-p_1)] / (p_1 - p_0)^2$. Fifty-two systemic lupus cases diagnosed as systemic lupus according to EULAR (2019) [14] have

been enrolled in the current research. Twenty-five gender and age-matched controls have been presented in our study.

Exclusion standard

Patients who have malignancies, pregnancy, diabetic nephropathy, other autoimmune diseases, and those with active infection were excluded.

2.2. Study design

The demographic data, complete history, and clinical exam have been evaluated for all cases. SLE activity was measured by utilizing SLEDAI [15]. Renal SLEDAI has been utilized to evaluate renal illness activity, with the score consisting of four renal-associated factors: pyuria, hematuria, proteinuria, and, finally, urine casts. Every one of them has a score of four. Patients were classified based on renal SLEDAI results. ALN has a renal SLEDAI more than four, while SLE patients with no renal activity (NRA) had a renal SLEDAI equal zero. [15]. Renal biopsies were performed in 17 cases with ALN in this research, and Renal biopsies have been categorized based on ISN and the Renal Pathology Society 2003 classification of Lupus Nephritis [16] and scored by the NIH chronicity and/ activity indices/ [17]. The greatest score was 24 points for (AI) and 12 for (CI). Full routine labs, immune profiles (ANA, Anti-ds DNA, RF, Anti-CCP, C3, and C4), and 24-hour urinary protein were done for patients. Our marker urine soluble CD163, was assessed in urine samples of patients by the ELISA technique.

2.3. Statistical Methods

Data has been gathered and encoded to enable modification, then input twice into Microsoft Access, with analysis conducted using version 22 of the Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, United States of America). Basic descriptive analysis using numerical values and percentages for qualitative information, together with arithmetic means as a measure of central tendency and standard deviations to assess the dispersion of quantitative parametric data. The quantitative data in this research have been first assessed for normalcy using the One-Sample Kolmogorov-Smirnov test for each group of study, after which inferential statistical tests have been used. For quantitative parametric data, an independent samples t-test has been utilized to compare the quantitative parameters among 2 independent groups. For quantitative non-parametric data, the

Mann-Whitney test is utilized to compare 2 independent groups. For qualitative information, the Chi-square test is utilized to compare 2 or more qualitative groups. Spearman's bivariate association test to assess the relationship among quantitative non-parametric parameters. Assessment of sensitivity and specificity for a novel test using the Receiver Operating Characteristic (ROC) curve. A *P*-value of less than 0.05 was deemed statistically significant.

3. Results

A case-control study included (46) females and (6) males with a mean age of (33.6±8.8 y). There were (27) with ALN and (25) NRA SLE cases, and (25) sex and age-matched healthy control subjects. Demographic and laboratory data of cases and controls are explained in Tables 1 and 2. The mean of urinary Cluster of Differentiation level 163 in cases (7±3.6) was elevated than in controls/ (4.4 ±1.03) with (P <0.001) as shown in figure 1. A significantly higher level of UsCD163 has been detected in ALN (9.3 ±3.5) than in NRA patients (4.5± 1.3) and controls (p < 0.001 in both), as in **Figure 2. Table 3** shows correlations of urinary soluble Cluster of Differentiation 163 with score SLEDAI (p =0.001), 24-hour urinary protein (p =0.001), C3 and C4 (p =0.001). **Table 4** showed that usCD163 had a discriminative power for differentiating SLE cases from controls, with the optimal cut-off value being (4.58 ng/ml), at which sensitivity is (75%) and specificity is (52%). Also, usCD163 has a good diagnostic ability to differentiate between cases of SLE that have active lupus nephritis and cases that have no renal activity, with an optimal cut-off value is (5.031 ng/ml), at which sensitivity is (88.9%) and specificity is (80%).

Table 1: baseline characteristics, clinical details, and treatment history of SLE cases with ALN and NRA.

Variables		ALN (n = 27)	NRA (n = 25)	*P*-value
Age (years)		34.3±9.7	32.8±7.8	0.53
Female: Male		23:4	25:2	0.67
Duration of disease		7.1±3.4	4.04±4.1	0.71
Score of SLEDAI		11.14±2.95	0.88±0.91	0.003
Medications	Methotrexate	0	2(8%)	0.22
	HCQ	26 (96.3%)	23 (92)	0.60
	Azathioprine	6 (22.2%)	8 (32%)	0.53
	Cyclophosphamide	11 (40.7%)	2 (8%)	0.01
	MMF	12 (44.4%)	3 (12%)	0.55
	Steroids(oral)	27 (100%)	22 (88%)	0.10
	IV Methylprednisolone	12(44.4%)	0	0.001

SLE: systemic lupus erythematosus, no-renal activity (NRA), hydroxychloroquine (HCQ), mycophenolate mofetil (MMF), active lupus nephritis (ALN), SLE disease activity index (SLEDAI).

Table 2: LAB examinations of SLE cases with ALN and NRA.

Variables	ALN (n = 27)	NRA (n = 25)	*P*-value
WBCs (mm3)	4.4 ± 1.6	5.2 ± 2.04	0.06
Platelets (mm3)	188.6 ± 67.7	229 ± 75.6	0.01
Serum Cr. (mg/dl)	0.76 ± 0.29	0.65 ± 0.17	0.10
AST (units per liter)	24.5 ± 7.2	23.3 ± 10.9	0.13
ALT (units per liter)	24.3 ± 14.2	22.5± 11.4	0.71
Proteinuria (g/24 h)	1.4±1.2	00.25±0.11	<0.001
+ve ANA	27	25	--
+ve anti-ds-DNA	24(88.9%)	8(32%)	<0.001
C3 level	82.3±15.6	114.1±21.7	<0.001
C4 level	10.8±5.9	18.3±6.4	<0.001
HB	10.1±0.86	11±1.4	0.006
ESR	60.9±23.7	41.7±25.2	<0.001
WBCs (mm3)	4.4 ± 1.6	5.2 ± 2.04	0.06
Platelets (mm3)	188.6 ± 67.7	229 ± 75.6	0.01
Serum Cr. (mg/dl)	0.76 ± 0.29	0.65 ± 0.17	0.10

white blood cells (WBCs), red blood cells (RBCs), erythrocyte sedimentation rate (ESR), amino transaminase (AST), creatinine (Cr.), antinuclear antibodies /{ANA}, anti-double stranded deoxyribonucleic acid antibody /{Anti-dsDNA antibody}, alanine aminotransferase (ALT), complement (C). *LN: Lupus Nephritis, ALN: Active Lupus Nephritis, SD: Standard Deviation.*

Table 3: Correlation between CD163 (ng/ml) with disease character variables among cases

Variables	CD163 (ng/ml)	
	R	P-value
Age (year)	-0.08	0.54
Disease duration (year)	0.07	0.60
Score of SLEDAI	0.57	0.001
Score of renal SLEDAI	-0.14	0.47
24 hrs protein (gm)	0.68	0.001
C3	-0.61	0.001
C4	-0.41	0.003
ESR	0.25	0.07
CRP titer	0.007	0.96

SLEDAI: *systemic lupus erythematosus,* ***C3****: complement 3,* ***C4****: complement 4,* ***ESR****: estimated sedimentation rate,* ***CRP****: C-reactive protein.*

Table 4: Sensitivity and specificity of CD163 level in the diagnosis of cases and subgroups.

Variable	Sensitivity	Specificity	AUC	Cut-off point	P-value (95% CI)
Cases & controls	75%	52%	75.5%	4.58	<0.001 (0.649- 0.861)
ALN & NRA	88.9%	80%	92.5%	5.031	<0.001 (0.857-0.994)

ALN: active lupus nephritis, NRA: no renal activity, AUC: area under the curve, CI: confidence interval.

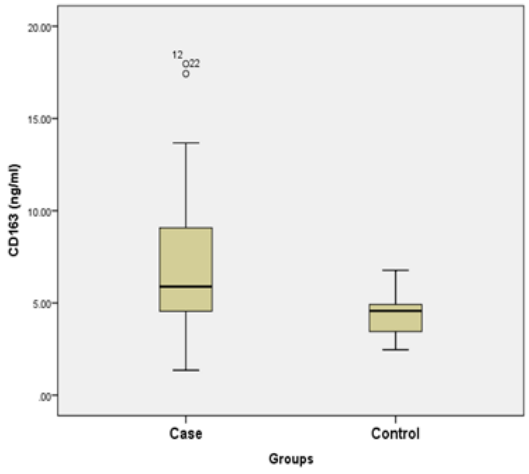

Figure 1: The difference in the level of usCD163 among cases and controls.

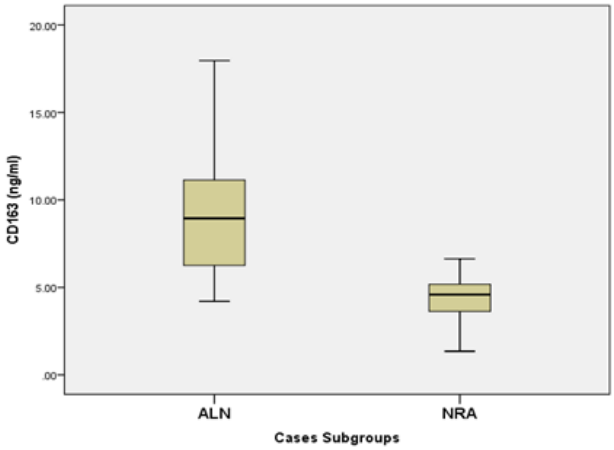

Figure 2: The difference in the level of usCD163 between ALN and NRA.

4. Discussion

LN is a dangerous and most prevalent clinical presentation of Systemic lupus (that is correlated with high death rate and morbidity [2]. Lupus nephritis is a form of GN that represents one of the critical symptoms of systemic lupus. Lupus nephritis is histologically categorized into 6 different categories that represent different symptoms and complications of kidney affection in systemic lupus [18]. Treatment of lupus nephritis is difficult, and it is essential to possess the ability to differentiate active nephritis from chronic kidney disease, as both usually present with impaired kidney function and proteinuria. Moreover, histologic findings and clinical findings are often discordant [19-21. Research has focused on urine sediment to find a novel lupus nephritis biomarker that potentially surmounts the constraints of existing ones. Macrophages were identified as the predominant urinary cells in the active sediment of lupus nephritis, then B and T cells [22]. Macrophages produce Cluster of Differentiation 163, which can be separated from the surface by proteolytic activity and then released into circulation as a soluble protein; hence, soluble Cluster of Differentiation 163 could be associated with the advancement of illness [23]. In this study, the urinary soluble CD163 has shown a higher level in ALN compared to NRA patients, with p >0.001. This outcome resembled research conducted by Huang et al.2022. The urine sCD163 is higher in cases with active LN in comparison to those with no renal activity and the control group. This outcome resembled research conducted by

Gamal et al.2022. These results suggest that in LN, M2 macrophages in the kidneys are activated locally, resulting in soluble CD163 synthesis via proteolysis, which is shown in urine. This can indicate a function for M2 macrophages in lupus nephritis etiology [24] and [8]. We found that usCD163 had a discriminative power for differentiating SLE cases from controls, with the optimal cut-off value being (4.58 ng/ml), at which sensitivity is (75%) and specificity is (52%). Moreover, usCD163 had a good diagnostic ability to differentiate between cases of SLE that have active lupus nephritis and cases that have no renal activity, with the optimal cut-off value is (5.031 ng/ml), at which sensitivity is (88.9%) and specificity is (80%). This outcome aligns with the research conducted by Gamal et al.2022. Also, urine soluble CD163 level is correlated with total SLEDAI and rSLEDAI with P <0.001, which is the same as several studies as Zhang et al 2020 and Huang et al 2022. Consequently, usCD163 may be regarded as a potential biomarker for assessing the illness flare of systemic lupus or lupus nephritis in the future [25]. Furthermore, urine soluble Cluster of Differentiation 163 levels significantly raise have been significantly raised in cases with positive anti-ds-DNA antibodies in comparison to those with negative findings, with a P value of 0.001.which was the same result found by the research done by Huang et al.2022.Also, a statistically significant negative correlation of the level of UsCD163 has been detected with level of C4, and C3 among cases with active lupus nephritis, which was the same result found by research done by Zhang et al 2020 and Huang et al 2022 and unlike the research of Gamal et al.2022 which detected insignificant correlation between amount of urine soluble CD163 and C3. We also found that a significant correlation has been detected between 24-hour urinary protein and urine soluble CD163 with a p <0. 001.This agrees with research performed by Zhang et al.2020. Urine soluble Cluster of Differentiation 163 may serve as a histological marker that aligns with the quantity of Cluster of Differentiation 163 cells invading the glomeruli. This association may enable urine-soluble Cluster of Differentiation 163 to distinguish between lupus nephritis categories characterized by a high density of infiltrating macrophages and those with lower inflammatory profiles [27]. We found that there was a numeric variation in the level of urine soluble CD163 related to different pathological classes of lupus

nephritis, but this variation showed a statistically insignificant variance with p >0.05 in CD163 level between different grades of renal biopsy findings. That was the same finding by a study done by Gamal et al.2022 unlike a study done by Gupta R.et al.2021 that showed the urine soluble amount was comparable in cases with proliferative lupus nephritis and those with membranous nephropathy within the active nephritis cohort. In contrast, several studies revealed that urine soluble CD163 was significantly higher in cases with proliferative lupus nephritis, especially in class IV [24, 26, 27]. The presentation of Cluster of Differentiation 163 on macrophages is affected by a lot of medications such as glucocorticoids, mycophenolate mofetil (MMF), cyclophosphamide, tacrolimus, and rituximab [9, 28, 29]. But, many researchers found that elevation in urine soluble Cluster of Differentiation 163 in active and proliferative lupus nephritis wasn't related to drugs like glucocorticoids and MMF Endo N et al.2016, Zhang et al.2020, and Gamal et al.2022.In this work, a statistically insignificant variance has been detected in the level of usCD163 among cases with active lupus nephritis in different types of treatments, with a p >0.05. The first limitation of our study is the cross-sectional type of study, so we didn't do serial usCD163 measurements, and long-term follow-up renal activities were not performed.

5. Conclusion

Urine soluble CD163 could be a measure of kidney illness activity in systemic lupus cases. It may distinguish between cases with ALN and SLE cases that do not have kidney activity. Its level is correlated with symptoms of systemic lupus and laboratory tests that show renal disease activity. Consequently, it serves as a potential indicator of LN activity.

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Authors' contributions: RAM: protocol development, data collection, and manuscript writing. SHS: manuscript analysis and editing, and final approval. RMA: manuscript revision and approval. YET: manuscript revision

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