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RESEARCH ARTICLE

Evaluation of Serum Cystatin C as a Marker of Glomerular filtration rate in Patients with Systemic Lupus Erythematosus.

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Abstract

Background: Lupus nephritis is one of the most serious manifestations in SLE and it occurs in about 60% of patients.

The objective of this study is to compare between serum cystatin C and cystatin C-based glomerular filtration rate (GFR) between SLE patients & control subjects, and between renal & non-renal SLE patients. Also to detect sensitivity & specificity of serum cystatin C as a marker of impaired GFR.
Methods: This study was carried out on 60 subjects, 30 SLE patients & 30 apparently healthy controls. Disease activity was assessed by Systemic Lupus Erythematosus Disease Activity Index SLEDAI-2k. Serum creatinine & creatinine-based GFR and serum cystatin C & cystatin C- based GFR were estimated.

Results: Serum cystatin C level was higher among the SLE patients than among the control group and the difference was highly significant (P = 0.000). No significant difference was found in serum cystatin C & cystatin C-based GFR among renal and non renal patients (P>0.05), and no significant difference was found in serum creatinine & creatinine- based GFR among renal & nonrenal patients. At a cut-off value of < 0.95mg/L, the sensitivity of serum cystatin C was 100% while specificity was only 5%.
Conclusion: Although the sensitivity of cystatin C was higher than that of creatinine, it will not add any more benefit in the routine assessment of GFR in SLE patients.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic inflammatory autoimmune disease that affects mostly all organs of the body (**Waldman and Appel, 2006**). Lupus nephritis (LN) is one of the most serious manifestations in SLE occurring in about 60% of patients (**Navaneethan et al., 2008**). It is a major cause of morbidity and mortality in patients with SLE (**El Bakry et al., 2014**).
The glomerular filtration rate (GFR) is the most important marker of renal function and crucial for diagnosis, stratification and response to treatment (**Soares et al., 2009**). There is a real need for a surrogate marker that can predict the degree of renal inflammation in SLE patients (**Li et al., 2006**). The ideal marker of GFR should be

constantly produced, be freely filtered, not reabsorbed or secreted by the renal tubules or metabolized or eliminated by extrarenal mechanisms (**Soares et al., 2009**).

Serum creatinine (SCr) is the most widely used parameter to assess glomerular filtration rate (GFR) in the last 40 y (**Stevens and Levey, 2005**). It is freely filtered by the glomerulus, not reabsorbed by the proximal tubules and is secreted in small amounts (**Rosner and Bolton, 2006**). Creatinine production varies considerably intra- and inter-individually. Tubular secretion of SCr increases when plasma concentrations increase. So, there is an overestimation of GFR in patients with mild to moderate decreases in GFR (**Stevens and Levey, 2005**). This non-linear relationship between its plasma concentration and GFRSCr makes it not sensitive for detecting small decreases in GFR (**Filler et al., 2002**).

CystatinC is produced at a constant rate by all body cells (**Stevens and Levey, 2005; Rosner and Bolton, 2006**). It has a molecular weight of 13.3 kDa. It is a cationic non-glycosylated protein which consists of a single polypeptide strand of 120 amino acids. Its main function is potent inhibition of cysteine proteinases (**Laterza et al., 2002**).

Cystatin C is freely filtered by the glomerulus, not returned to the bloodstream and not secreted by the renal tubules (**Madero, et al., 2006**). The above features make it, theoretically, a better marker of renal function than creatinine (**Seronie-Vivien et al., 2008**).

The aim of this study is to compare serum cystatin C and cystatin C-based glomerular filtration rate among SLE patients & control subjects, and among renal & nonrenal SLE patients. We aimed, also, to detect the sensitivity & specificity of serum cystatin C as a marker of impaired GFR.

Materials and methods

This study was conducted on 60 subjects; 30 patients randomly selected from the outpatient clinics, inpatient departments and follow-up units of Rheumatology and Rehabilitation & Dermatology, Venereology and Andrology Departments, Faculty of medicine, Zagazig University hospitals (Egypt) and 30 apparently healthy subjects as controls. The subjects were divided into two groups. The first group (I): included 30 patients suffering from SLE, diagnosed according to the revised American College of Rheumatology (ACR) criteria for classification of SLE (**Hochberg, 1997**). The second group (II): included 30 apparently healthy subjects, matched for age and sex, taken as a control group.

In this study, we excluded patients with renal failure, those with any renal disease prior to the onset of SLE, patients with thyroid dysfunction (hypothyroidism / hyperthyroidism) or patients taking high dose of glucocorticoids.

The study was approved by the local medical ethical committee of Faculty of Medicine, Zagazig University and an informed consent was obtained from each participant in the study.

All subjects of our study were subjected to full history taking, thorough physical examination, assessment of disease activity was done by using systemic lupus erythematosus disease activity index-2k (SLEDAI-2k) (**Gladman at al., 2000**), and laboratory investigations such as: complete blood count (CBC) by sysmex Kx-21 (Japan), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP)by Integra 400 plus (Roche, Germany), complete urine analysis, 24-h urinary proteins, serum urea &creatinine, by Dimension RXL-Max (Siemens, USA),antinuclear antibody (ANA) , anti-double stranded DNA (Anti-Ds. DNA) by immunofluorescent assay.

Venous blood samples were taken from the subjects. The serum was separated from the cellular fraction, stored in -20^0 C, and the serum cystatin C levels were measured by an Enzyme Linked Immunosorbent Assay (ELISA) kit (BioVendor- Laboratornimedicinaa.s.). The intra-assay coefficients of variation for the cystatin C measurement was 3.1% and the inter-assay coefficients of variation was 3.4%.

Glomerular filtration rate(GFR) was assessed for each patient by 2 different equations: one by using serum creatinine level and the other by using serum cystatin level.

GFR was estimated using the re-expressed four variables MDRD equation (**Levey et al., 1999**).

$GFR = 175 \times \text{serum creatinine (mg/dl)}^{(-1.154)} \times \text{age (years)}^{(-0.203)} \times 0.742$ (if female)

We used Hoek equation for estimation of GFR using serum cystatin level (**Hoek et al., 2003**):

GFR ml/min/1.73 m2 = - 4.32 + (80.35 × 1/cystatin C)

Results

Patients involved in this study had SLEDAI ranging from 2 to 28. The most prominent clinical findings were arthralgia/arthritis & malar rash present in 23 (76.7%) and 20 (66.7%) patients respectively (table 2). Laboratory findings denoted that major organ involvement in this studied group was renal involvement, present in 21 pts (70%). The demographic & laboratory characteristics of SLE patients are shown in table (1).

Serum cystatin C was higher among SLE patients than among the control group, while cystatin C-based GFR was lower in SLE patients than the control subjects (table 3), and the difference was highly significant for both of them ($t = 9.6$ & $t = -9.13$ respectively, $P < 0.01$).

Table (4) shows no correlation between neither serum creatinine nor serum cystatin C with age, SLEDAI, ESR and CRP where all correlations were insignificant ($P > 0.05$ for all).

No significant difference was found in serum cystatin & cystatin-based GFR among renal and non renal patients ($P>0.05$), at the same time no significant difference was found in serum creatinine & creatinine- based GFR among renal & nonrenal patients (table 5).

At serum cystatin C cut-off value of ≤ 0.95mg/L, sensitivity was 100% while specificity was only 5% (using creatinine-based GFR as a standard test) (table 6). The positive predictive value (PPV) was 34.5 % and the negative predictive value (NPV) was 100 %.

Table (1): Quantitative demographic & laboratory characteristics of SLE patients

SLE patients (n=30)	Median	Range
Age(yrs)	32	23-40
Disease duration(yrs)	4	0.5-14
Protein in 24 hr urine (gm/24hr)	406.5	90-3392
Serum urea *(mg/dl)*	11	6-27
Serum creatinine(mg/dl)	0.7	0.3-1.4
Creatinine based GFR (ml/min)	100	44-255

Table (2): Frequencies of SLE manifestations in the study population

	No	%
Proteinuria (>0.5gm/24hrs)	13	43.3
Casts (Heme-granular/red cell)	14	46.6
Hematuria (>5RBC/HPF)	12	40
Pyuria (> 5 WBC/HPF)	16	53.3
Malar rash	20	66.7
Discoid rash	0	0
Photosensitivity	11	36.7
Arthralgia/arthritis	23	76.7
Oral ulcers	19	36.3
Hair loss	9	30
Pleuritis	6	20
Pericarditis	1	3.33
Seizures	0	0
Psychosis	0	0
Organic brain disorder	0	0
Cranial nerve	1	3.3
Visual disturbance	1	3.3

Lupus Headache	0	0
Muscle involvement	1	3.3
Fever	6	19.4
Hemolytic anemia	0	0
Leucopenia	4	13.3
Thrombocytopeia	6	20
ANA	26	86.7
Anti-dsDNA	18	60

ANA: antinuclear antibody, ds-DNA: double-stranded DNA

Table (3): Difference between serum cystatin C & cystatin C-based GFR in SLE patients and control subjects

	SLE group (n=30)	**Control group (n=30)**	**T**	**P**
Serum Cystatin C (mg/L) (Mean ± SD)	1.125± 0.22	0.68 ± 0.09	9.6	0.000
Cystatin C-based GFR (ml/min/1.73 m2) **(Mean ± SD)**	70.034 ± 17.35	116.469 ± 17.79	-9.13	0.000

GFR: glomerular filtration rate

Table (4): Correlations between serum creatinine and serum cystatin C with some non-renal factors

Correlations	***Serum creatinine***		***Serum cystatin C***	
	r	***P***	***R***	***P***
Age	*-0.301*	*0.210*	*-0.017*	*0.945*
SLEDAI	*0.128*	*0.603*	*-0.312*	*0.193*
ESR	*0.023*	*0.926*	*0.006*	*0.981*
CRP	*0.111*	*0.651*	*0.154*	*0.529*

SLEDAI: SLE disease activity index, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein.

Table (5): Difference of serum creatinine, serum cystatin C and their GFR-based equations among renal & non-renal SLE patients

	Renal (n=21) Median (range)	Non-renal (n=9) Median (range)	MW	P
Serum Creatinine (mg/dl)	0.7 (0.3-1.4)	0.6 (0.5-0.9)	71	0.279
Serum Cystatin C (mg/L)	1.1 (1-1.55)	1 (0.54 -1.48)	63.5	0.156
Creatinine-based GFR	97.6 (44.2- 254.7)	112.5 (73.5-197.9)	70.5	0.277
Cystatin C- based GFR	68.73 (47.5-76)	75.63 (50.15-144.48)	63.5	0.156

**Mann Whitney U test was used*

Table (6): Sensitivity & specificity of serum cystatin C

	Creatinine-based GFR		
	Low GFR (< 90ml/min)	***Normal GFR (> 90ml/min)***	*Total*
High cystatin C (> 0.95mg/L)	*10*	*19*	*29*
Normal cystatin C (< 0.95mg/L)	*0*	*1*	*1*
Total	*10*	*20*	*30*

Sensitivity: 100%
Specificity: 5%

Predictive Values

- ***PPV: 34.5***
- ***NPV: 100***

Discussion:

Serum cystatin C has been considered as a potential candidate to replace serum creatinine in the estimation of glomerular filtration rate (**Kos et al., 1998**). Many studies claimed serum cystatin C as a reliable marker of GFR in patients with impaired kidney function having a higher diagnostic accuracy than serum creatinine in some studied groups (**Hojs et al., 2006**). However, several studies have shown cystatin C to be still questionable due to the variability in the relationship between GFR and serum cystatin C among the different populations evaluated (**Madero et al., 2006**). This variability has been attributed to differences in its generation and tubular reabsorption among different individuals (**Stevens at al., 2008**). Moreover, other researchers attributed this variability to different factors other than renal factors like its relation to inflammation **(Lertnawapan et al., 2012)**, atherosclerosis **(Imai et al., 2011)**, corticosteroid use **(Grubb, 2001)** and other factors.

Cystatin C use in glomerular filtration rate estimation has been studied in different populations like those with malignancy **(Al-Tonbary et al., 2004)**, cirrhotic liver **(Chung et al., 2010),** diabetes mellitus (**Jeon et al., 2013**), HIV patients **(Gagneux-Brunon et al., 2013)** and transplant patients **(Ayub et al.,2013)**. In this study we decided to evaluate cystatin C in a group of SLE patients as renal involvement is one of the most important causes of morbidity & mortality in SLE to see what cystatin C can add to renal assessment and follow up.

In our study, serum cystatin C were significantly higher in SLE patients compared to controls, while cystatin-based GFR were significantly lower in SLE patients than controls ($t=9.6$ & $t=-9.13$ respectively, $p< 0.01$). These findings are in accordance to the study of **Lertnawapan et al. (2012)**, and **Chew et al. (2013)**, in which serum cystatin C was also found to be higher in SLE patients compared to controls.

Comparing serum creatinine & serum cystatin C as regards to their relation with multiple non-renal factors like age, SLEDAI, ESR, and CRP revealed that both were not related to any of these factors in our SLE patients (all with $P>0.05$). Even the influence of age on both creatinine **(Glassock , 2009)** and cystatin C **(Knight et al., 2004)** was not clear in this studied group since that most SLE patients were in a similar age group, being a disease that selectively affects females in the childbearing period. Many studies were conducted to evaluate the influence of these non-renal factors on cystatin C, some agreeing with our findings and some disagreeing (**Lertnawapan et al., 2012**; **Wasén et al., 2008**), making the influence of these factors still unconfirmed. Away from the conflicting results between different studies, these findings helped us to compare both creatinine and cystatin as markers of renal impairment independent of all previously mentioned factors in this group of SLE patients.

The comparison between renal & nonrenal SLE patients as regards serum creatinine & creatinine-based GFR showed no significant difference between the two groups ($P>0.05$ for both). But, also serum cystatin C & cystatin C-based GFR were very disappointing since that no significant difference was found between renal & nonrenal patients ($P>0.05$ for both), denoting that serum cystatin C & cystatin C-based GFR were so much similar to serum creatinine & creatinine-based GFR with no superiority of cystatin C over creatinine in detecting patients with nephritis or early renal damage without impairment of renal function. Although these results differ from that of **Coll et al.,** who found that serum cystatin C reflects GFR changes more rapidly compared to serum creatinine (**Coll et al., 2000**) and the study of **Star et al.,(2002)** who also found that Cystatin C was a superior GFR marker to creatinine in chronic renal insufficiency. Our results were similar to the results of **Bouvet et al. (2006**) and **Eriksen et al.** (**2010**) who found that cystatin C was not a better estimator of GFR with no superiority over creatinine, and went to the conclusion that GFR is better estimated by considering simultaneous estimation of more than one biomarker.

Using serum cystatin C of 0.95 mg/L as a cut off value; the sensitivity of cystatin C was very high reaching 100%, but its specificity was very low about 5%. These results were very much different from the results of **Narvaez-Sanchez et al.(2008)** who found that cystatin C sensitivity was 75% & the specificity was high being 84%, while our results were similar to the results of **Zhang et al.(2013)** who conducted a meta-analysis for the evaluation of cystatin and stated that the diagnostic sensitivity of Cystatin C was high even higher than that of serum creatinine, but the diagnostic specificity was lower with cystatin C. So the question remains: Will cystatin C ever be considered reliable enough to replace creatinine as a standard measure in the estimation of glomerular filtration rate?

The limitations & recommendations of our study included the following points: First; although cystatin C concentrations have been shown to be uninfluenced by race (**Lee et al., 2000)** all subjects included in this study were Caucasians no other races were included making it necessary to test SLE patients from different races to confirm this. Second; we did not study the influence of sex and steroid use on cystatin C since that in our patients only 2 patients were males and only 2 patients were steroid non-users **(Silva et al., 2011)**. Third; we measured cystatin C only once in each subject so we could not confirm the presence or absence of intra-patient variability in serum cystatin C that was previously documented **(Keevil et al., 1998),** which is a point that requires further assessment since that some studies like the study of **Podracka et al. in 2005** on transplant patients denied it. Fourth; in spite of the high sensitivity of cystatin C, the number of false positive cases was high denoting the necessity of readjusting cystatin C cut off values to obtain better sensitivity & specificity (**Podracka et al., 2005**).

Finally, we conclude that cystatin C was not superior to creatinine in detecting patients with nephritis or early renal damage without impairment of renal function. We also suggest that although the sensitivity of cystatin-C was very high it will not add any more benefit in the routine assessment of glomerular filtration rate in SLE patients putting into consideration its low specificity & high price compared to creatinine. Also we assume that even if its price & its use becomes readily available, it would be wiser to use GFR equations that are based on both creatinine & cystatin C for better accuracy.

Conflict of interest

The authors have no conflict of interest to declare.

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