

Human Epididymis Protein 4: A Novel Biomarker for Lupus Nephritis and Chronic Kidney Disease in Systemic Lupus Erythematosus

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Background: Human epididymis protein 4 (HE4) is an available tumor biomarker for detecting ovarian cancer. However, it is unknown if serum HE4 could be a novel biomarker for diagnosis of lupus nephritis (LN) and chronic kidney disease (CKD) in patients with systemic lupus erythematosus (SLE). **Methods:** This study enrolled 209 SLE patients, 75 patients with renal dysfunction without SLE and 32 healthy subjects. HE4 concentrations were analyzed by ELISA (enzyme-linked immunosorbent assay; Fujirebio Diagnostics, Sweden). The receiver operating characteristic (ROC) curves were constructed to assess diagnostic accuracy of HE4 for LN or CKD in SLE. **Results:** Serum HE4 level was significantly higher in SLE patients than that in healthy controls ($P < 0.001$), especially for

those with LN or CKD. It was also higher in patients with renal dysfunction without SLE than healthy controls ($P < 0.001$), while there was no significant difference between these patients and those with SLE with CKD ($P = 0.73$). Multivariate analysis showed significant association between increased HE4 and LN or CKD after controlling for confounders. ROC curves showed the cutoff values were 150.1 pM (sensitivity, 76.8%; specificity, 91.1%) for the diagnosis of LN in SLE and 233.9 pM (sensitivity, 92.9%; specificity, 93.5%) for CKD in SLE. **Conclusions:** Increased serum HE4 level is closely associated with the development of LN or CKD in SLE patients. Furthermore, it can be used as a novel and useful biomarker for diagnosis of LN or CKD. *J. Clin. Lab. Anal.* **30**:897–904, 2016. © 2016 Wiley Periodicals, Inc.

Key words: human epididymis protein 4; nephritis; chronic kidney disease; systemic lupus erythematosus

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease of unknown etiology, characterized by multiple organ involvements. Lupus nephritis (LN) is a common manifestation and one of the most serious organ involvements, affecting 50–70% of SLE patients (1). LN patients often have a low 5-year survival (2), although the course of renal disease can be dramatically modified by early diagnosis and prompt treatment, which contributes to the improvement of long-term survival (3). Therefore, it is of great importance to detect LN in SLE at an early stage.

The majority of SLE studies focused on renal failure or remission achievement in LN patients, whereas less attention was paid to the presence or absence of chronic kidney

disease (CKD) in SLE patients (4–8). Actually, CKD is closely associated with mortality and cardiovascular disease as well as the development of end-stage renal disease

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(ESRD) (9). Therefore, an accurate and prompt identification for CKD is of great significance to help clinicians improve clinical outcome and prevent the development of ESRD and cardiovascular disease as well as the death in SLE, especially for the patients with LN.

Human epididymis protein 4 (HE4), first identified and characterized as a human epididymis-specific protein in 1991 (10), is widely detected in respiratory tract, nasopharynx, salivary glands, kidney, and other organs (11–13), although several subsequent studies demonstrated the clinical value of HE4 alone or in combination with CA125 for diagnosing and predicting ovarian cancer (14–19). Recently, significant increment of serum HE4 concentration has also been found in CKD, renal failure, and heart failure (20–23). Nonetheless, the diagnostic efficacy of serum HE4 has not been explored in SLE, especially for those with LN or CKD. The aim of this study is to determine whether serum HE4 could be a novel and useful biomarker for diagnosis of LN and CKD in SLE patients.

MATERIALS AND METHODS

Two hundred nine SLE patients (184 women and 25 men, mean \pm SD, age 39 ± 16 years) were consecutively recruited from Changzheng Hospital between August 2008 and July 2013. All the patients fulfilled at least four of the 1997 revised American College of Rheumatology (ACR) classification criteria for SLE (24). Of those, 138 patients (120 women and 18 men, mean \pm SD, age 38 ± 16 years) fulfilled ACR criteria for LN (25), and 70 patients (62 women and 8 men, mean \pm SD, age 43 ± 16 years) had CKD according to the kidney disease: improving global outcome definition (estimated glomerular filtration rate [eGFR] < 60 ml/min/1.73 m² of body surface area for 3 months or more) (26). Seventy-five patients with renal dysfunction without SLE (mean \pm SD, age 53 ± 17 years; female/male: 66/9), and 32 healthy subjects (mean \pm SD, age 40 ± 8 years; female/male: 29/3) with no histories of SLE or other autoimmune or inflammatory diseases were randomly recruited. Of the controls, the subjects were excluded if they had abnormal renal function (eGFR < 90 ml/min/1.73 m²), tumor, or gynecological diseases.

Some clinical and demographic characteristics and laboratory data of the patients were obtained by medical record review. The SLE disease activity index (SLEDAI) score (27) was determined for each patient at the day when blood was drawn for laboratory tests. According to the physical and radiological examination, no ovarian disorders were detected in the study cohorts. Creatinine, urea, and uric acid (UA) were measured by enzymatic assay (Cobas c701, Roche). eGFR was calculated by chronic kidney disease epidemiology collaboration (CKD-EPI) equation, which is a newly developed and validated

formula for eGFR. It is more accurate at normal or near-normal eGFR ($eGFR_{CKD-EPI} = 141 \times \min [Scr/\kappa, 1]^\alpha \times \max [Scr/\kappa, 1]^{-1.209} \times 0.993^{Age} \times [1.018 \text{ if female}] \times [1.159 \text{ if black}]$, where Scr is serum creatinine, κ is 0.7 for females and 0.9 for males, α is -0.329 for females and -0.411 for males, min indicates the minimum of Scr/ κ or 1, and max indicates the maximum of Scr/ κ or 1) (28,29).

Serum samples were prepared immediately by centrifugation of peripheral venous blood and processed with cryopreservation (-80°C) for determination of HE4 level. Serum HE4 concentration was measured in duplicate by enzyme-linked immunosorbent assay (ELISA; Fujirebio Diagnostics, Sweden), with a measuring range of 15–900 pM. The inter- and intra-assay variation were $< 7\%$ and $< 3\%$, respectively. The samples with HE4 concentrations over 900 pM were measured again after dilution with HE4 calibrator by 1/10 according to the instructions.

Statistical Analysis

Data analyses were performed using SPSS 17.0 statistical software. Since all the continuous variables but age showed a skewed distribution, median and range were used to describe the levels of these variables. Differences between groups were compared using Mann–Whitney test for continuous variables. Spearman's correlation coefficient (r_s) was used to evaluate the correlation between HE4 and creatinine and eGFR. *P*-value less than 0.05 was considered as statistically significant. Receiver operating characteristics (ROCs) curves were constructed to assess sensitivity, specificity, and areas under the curves (AUCs) with 95% confidence interval (CI). In accordance with a previous study, the optimum cutoff value for diagnosis was selected by maximizing the sum of sensitivity and specificity and minimizing the overall error (square root of the sum $[1 - \text{sensitivity}]^2 + [1 - \text{specificity}]^2$), and by minimizing the distance of cutoff value to the top-left corner of the ROC curve (30).

RESULTS

Comparison of HE4 Levels Between Various Subgroups

The median level of serum HE4 was 166.3 pM (range, 16.7–7084.9) in the SLE group, significantly higher than that in the control group (median, range: 40.6 pM, 34.5–61.1) ($P < 0.001$). Then SLE patients were divided into four groups, namely SLE without LN or CKD ($n = 67$), SLE only with LN ($n = 72$), SLE only with CKD ($n = 4$), and SLE with LN and CKD ($n = 66$). The median levels of serum HE4 were 64.3 pM (range: 16.7–425.7 pM), 161.4 pM (range: 42.8–1218.6 pM), 282.7 pM (range: 183.0–1399.5 pM), and 613.8 pM (range: 156.4–7084.9 pM)

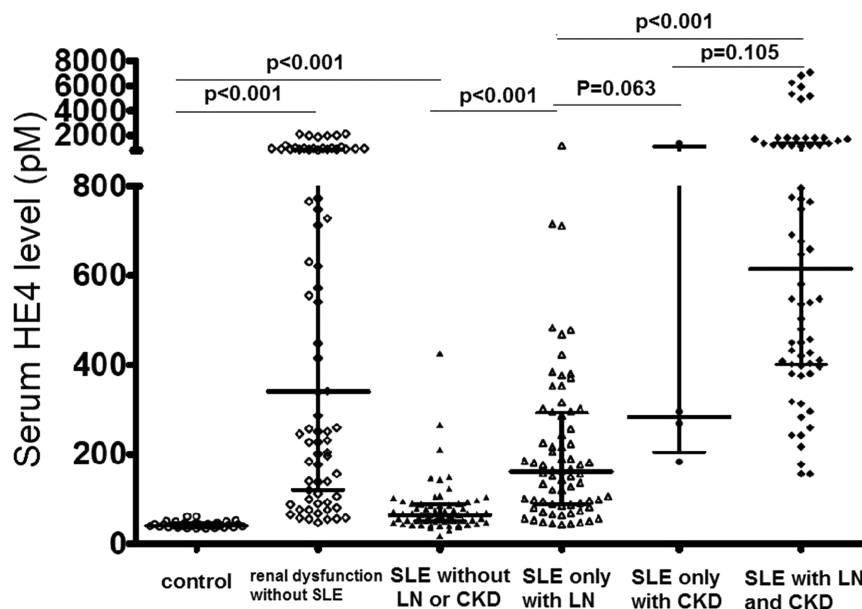


Fig. 1. Serum HE4 levels in controls and various SLE subgroups.

in SLE without LN or CKD, SLE only with LN, SLE only with CKD, and SLE with LN and CKD groups, respectively. All SLE subgroups showed significantly higher HE4 levels than the healthy control group. Compared with SLE without LN or CKD subgroup, others showed significantly increased HE4 levels. Especially SLE with LN and CKD subgroup showed significantly higher HE4 level than SLE only with LN subgroup. Serum HE4 level was also significantly higher in patients with renal dysfunction without SLE (median, range: 339.9 pM [47.3–2140]) than healthy controls ($P < 0.001$), while there was no significant difference between these patients and those with SLE only with CKD ($P = 0.73$; Fig. 1).

Association of Various Variables With LN or CKD in SLE Patients

Compared with SLE patients without LN, those with LN were significantly more likely to have previously documented hypertension ($P = 0.008$), positive anti-dsDNA antibody ($P < 0.001$), anemia ($P < 0.001$), leukopenia ($P = 0.017$), low C3 ($P < 0.001$), lower IgG ($P = 0.001$) and IgM ($P = 0.018$), higher SLEDAI score ($P < 0.001$), and less likely to have anti-SSA positive antibody ($P = 0.039$). However, other variables were not associated with LN (Table 1).

Next, by inclusion of these significant variables and HE4, multivariate logistic regression was used to examine whether or not HE4 was independently associated with LN in SLE patients. The results indicated that after controlling for above-mentioned confounders, only HE4 and

SLEDAI score remained significantly positive, but IgG inverse associations with LN (Table 2).

We also examined the association between various variables and CKD in SLE patients. We found that CKD patients were significantly more likely to have previously documented hypertension ($P = 0.001$), positive anti-dsDNA antibody ($P = 0.021$), anemia ($P < 0.001$), LN ($P < 0.001$), higher SLEDAI score ($P < 0.001$), and lower IgM ($P = 0.013$). However, other variables were not associated with CKD (Table 1).

Also, multivariate logistic regression was used to examine whether or not HE4 was independently associated with CKD development in SLE patients by including these significant variables. The results suggested that after controlling for above-mentioned confounders, only HE4 was significantly associated with CKD (Table 2).

Diagnostic Performance of HE4 for LN or CKD in SLE Patients

The median concentration of serum HE4 in SLE with LN patients ($n = 138$) was 317.1 pM (range: 42.8–7084.9), significantly higher than that in SLE without LN patients (median, range: 67.8 pM, 16.7–1399.5) ($P < 0.001$). ROC curves were used to investigate the diagnostic performance of serum HE4 for LN in 209 SLE patients, compared with that of serum creatinine, urea, and UA, which have been confirmed to be useful indicators for kidney diseases. Serum HE4 had significantly better diagnostic performance with 150.1 pM of optimum diagnostic cut-off value (AUC = 0.878, 95% CI: 0.829–0.926, sensitivity 76.8%, specificity 91.1%) for LN in SLE patients than did

TABLE 1. Clinical and Laboratory Characteristics of SLE Patients With LN or CKD Compared Without LN or CKD, Respectively

	SLE with LN (<i>n</i> = 138)	SLE without LN (<i>n</i> = 71)	<i>P</i> -value	SLE with CKD (<i>n</i> = 70)	SLE without CKD (<i>n</i> = 139)	<i>P</i> -value
Age (mean ± SD, years)	38 ± 16	40 ± 16	0.174	43 ± 16	37 ± 15	0.174
Sex (Female/male)	120/18	64/7	0.502	62/8	123/16	0.986
Disease duration (months, range)	12 (0.1–312)	19 (0.3–276)	0.149	24 (0.1–312)	12 (0.1–276)	0.156
History of hypertension (% , <i>n</i>)	25.4 (35)	9.9 (7)	0.008	34.3 (24)	14.4 (20)	0.001
History of diabetes (% , <i>n</i>)	0.7 (1)	5.6 (4)	0.085	0 (0)	3.6 (5)	0.260
Current prednisolone (% , <i>n</i>)	68.8 (95)	67.6 (48)	0.856	71.4 (50)	67.6 (94)	0.575
Current cyclophosphamide (% , <i>n</i>)	18.8 (26)	18.3 (13)	0.926	22.9 (16)	16.5 (23)	0.269
Anti-dsDNA antibody positive (% , <i>n</i>)	52.9 (73)	27.8 (19)	<0.001	55.7 (39)	38.8 (54)	0.021
Anti-SSA antibody positive (% , <i>n</i>)	57.2 (79)	71.8 (51)	0.039	58.5 (41)	64.0 (89)	0.443
Anti-SSB antibody positive (% , <i>n</i>)	20.3 (28)	22.5 (16)	0.706	15.7 (11)	23.7 (33)	0.179
Anti-U1RNP antibody positive (% , <i>n</i>)	34.1 (47)	39.4 (28)	0.443	31.4 (22)	38.1 (53)	0.340
Anti-Smith antibody positive (% , <i>n</i>)	25.4 (35)	28.2 (20)	0.663	24.3 (17)	27.3 (38)	0.636
Anemia (% , <i>n</i>)	56.5 (78)	25.4 (18)	<0.001	77.1 (54)	30.9 (43)	<0.001
Leukopenia (% , <i>n</i>)	40.6 (56)	23.9 (17)	0.017	37.1 (26)	33.8 (47)	0.634
Thrombocytopenia (% , <i>n</i>)	26.1 (36)	22.5 (16)	0.574	31.4 (22)	21.6 (30)	0.120
Low C3 (% , <i>n</i>)	92.0 (127)	69.0 (49)	<0.001	88.6 (62)	82.0 (114)	0.220
Low C4 (% , <i>n</i>)	90.5 (125)	84.5 (60)	0.192	82.9 (58)	91.4 (127)	0.069
IgG (g/l)	11.0 (1.2–47.2)	14.9 (2.1–48.1)	0.001	12.0 (2.07–48.10)	5.4 (1.9–47.2)	0.445
IgM (g/l)	0.88 (0.09–5.45)	1.24 (0.15–9.70)	0.018	0.87 (0.15–5.45)	1.06 (0.09–9.70)	0.013
IgA (g/l)	2.31 (0.09–6.79)	2.39 (0.55–6.42)	0.341	2.33 (0.09–5.67)	2.32 (0.55–6.79)	0.341
ESR (mm/hr)	44 (3–150)	38 (6–150)	0.305	48 (6–150)	38 (3–150)	0.305
HsCRP (mg/l)	3.08 (0.2–283)	4.17 (0.75–92.8)	0.078	4.47 (0.22–72.48)	4.17 (0.20–283)	0.444
SLEDAI score	13 (4–31)	7 (2–20)	<0.001	13 (4–31)	9 (2–30)	<0.001
LN (% , <i>n</i>)	/	/	/	94.3 (66)	51.8 (72)	<0.001

LN, lupus nephritis; SLE, systemic lupus erythematosus; CKD, chronic kidney disease; SD, standard deviation; SLEDAI, SLE disease activity index.

serum creatinine (AUC = 0.827, 95% CI: 0.771–0.883, sensitivity 74.6%, specificity 76.1%), urea (AUC = 0.818, 95% CI: 0.761–0.875, sensitivity 60.1%, specificity 94.4%), and UA (AUC = 0.803, 95% CI: 0.742–0.863, sensitivity 75.4%, specificity 77.5%), especially in SLE patients without CKD (AUC, 95% CI; HE4 vs. creatinine vs. urea vs. UA: 0.818, 0.747–0.889 vs. 0.716, 0.630–0.801 vs. 0.700, 0.614–0.787 vs. 0.724, 0.640–0.808) and in those with normal eGFR (AUC, 95% CI; HE4 vs. creatinine vs. urea vs. UA: 0.778, 0.689–0.867 vs. 0.681, 0.578–0.784 vs. 0.649, 0.544–0.754 vs. 0.733, 0.638–0.828; Fig. 2 and Table 3).

Serum HE4 level in SLE with CKD patients (*n* = 70) was 564.2 pM (range: 156.4–7084.9), significantly higher than that was in SLE without CKD patients (median, range: 89.1 pM, 16.7–1218.6) (*P* < 0.001). Since serum creatinine was used to calculate eGFR, its diagnostic performance for CKD was not assessed by an ROC curve. However, correlations between HE4 and creatinine (r_s = 0.638, *P* < 0.001) and eGFR (r_s = –0.604, *P* < 0.001) were statistically significant in SLE with CKD patients (Fig. 3). ROC curves showed the optimum cutoff value of diagnosing CKD in SLE was 233.9 pM (AUC = 0.948, 95% CI: 0.922–0.975, sensitivity 92.9%, specificity 93.5%), 10.25 mmol/l (AUC = 0.920, 95% CI: 0.877–0.963, sensitivity 81.4%, specificity 89.9%), and 326.5 μmol/l (AUC = 0.857, 95% CI: 0.798–0.916, sensitivity 81.4%, speci-

ficity 79.9%) for HE4, urea, and UA, respectively. Moreover, in SLE patients with CKD4–5 (eGFR < 30 ml/min/1.73 m²), the AUC was 0.959 (95% CI: 0.934–0.984) with sensitivity of 97.1% and specificity of 84.5%, 0.944 (95%CI: 0.904–0.985) with sensitivity 91.4% and specificity 86.2% and 0.847 (95% CI: 0.776–0.917) with sensitivity 74.3% and specificity 85.1%, respectively, for HE4, urea, and UA. Also, we investigated the diagnostic performance of HE4 for CKD in LN patients. Similarly, HE4 showed a great value for distinguishing CKD from LN patients (Fig. 4 and Table 3).

DISCUSSION

This is, to our knowledge, the first study to investigate the association of serum HE4 level with LN or CKD in SLE patients. In this study, we found that increased serum HE4 is independently associated with the development of LN or CKD in SLE patients. Furthermore, HE4 could be used as a novel and valuable indicator for diagnosis of LN or CKD in SLE.

Although proteinuria, serum creatinine, C3, C4, anti-dsDNA antibody, etc. has currently been used to screen and monitor LN development in SLE patients, their individual predictive value is modest (31). The diagnosis of LN needs to be confirmed by a renal biopsy. However,

TABLE 2. Multivariate Logistic Regression of the Relationship of Risk Factors With LN or CKD in SLE Patients

Risk factors	OR (95% CI)	P-value
LN		
History of hypertension (yes, no)	2.979 (0.873–10.167)	0.081
Leukopenia (yes, no)	1.614 (0.617–4.223)	0.330
Anemia (yes, no)	1.402 (0.497–3.956)	0.523
Low C3 (yes, no)	2.577 (0.779–8.528)	0.121
IgG(g/l)	0.916 (0.861–0.975)	0.006
IgM (g/l)	0.865 (0.600–1.248)	0.438
Anti-dsDNA antibody (yes, no)	1.746 (0.694–4.394)	0.236
Anti-SSA antibody (yes, no)	0.639 (0.258–1.584)	0.333
SLEDAI score (per)	1.313 (1.157–1.489)	<0.001
HE4 (per pM)	1.005 (1.001–1.008)	0.005
CKD		
History of hypertension (yes, no)	2.510 (0.859–7.338)	0.093
Anemia (yes, no)	2.227 (0.840–5.899)	0.107
IgM (g/l)	0.476 (0.215–1.051)	0.066
Anti-dsDNA antibody (yes, no)	2.387 (0.862–6.611)	0.094
SLEDAI score (per)	0.980 (0.895–1.073)	0.662
LN (yes, no)	2.019 (0.458–8.906)	0.353
HE4 (per pM)	1.007 (1.004–1.010)	<0.001

LN, lupus nephritis; SLE, systemic lupus erythematosus; CKD, chronic kidney disease; SLEDAI, SLE disease activity index.

as an invasive procedure, repeated renal biopsies are not always accepted by patients. Moreover, the global kidney status cannot be reliably reflected by every renal biopsy. Accordingly, it is of great importance to identify a noninvasive biomarker that can early differentiate LN especially before obvious structural renal damage and loss of renal function from SLE for providing a window to treat early LN and obviate severe renal tissue injury. In current study, we found that serum HE4 level in LN patients is significantly higher than that in SLE patients who show even

higher HE4 level than healthy controls do. Next, our multivariate analysis showed that HE4, IgG, and SLEDAI score are closely independently associated with LN development in SLE patients. As can be expected, serum IgG inversely correlates with LN, since an amount of IgG is consumed to form glomerular deposition of immune complexes. The association of SLEDAI score with LN is also expected as it is a standard parameter used for defining active LN. Interestingly, our data demonstrated no independent association of complement and anti-dsDNA antibody with LN development. In fact, it has been widely reported that they are not specific and could not be used as optimal biomarkers for LN (32–36).

Next, our results showed a significant diagnostic performance of serum HE4 for LN. Furthermore, the performance is even better than that of serum creatinine, urea, and UA. Especially in SLE patients without CKD or with normal eGFR, the AUCs for HE4 still reached 0.818 and 0.778, respectively, although they were decreased, compared with those in total SLE patients. In contrast, the AUCs for serum creatinine, urea, and UA are below 0.75 in SLE patients without CKD or with normal eGFR. These results suggested that serum HE4 could be a promising, useful biomarker for early diagnosis of LN, especially before obvious structural renal damage and loss of renal function, in SLE patients.

Also, we explored, in this study, the association of serum HE4 with CKD in SLE patients. Our data demonstrated that HE4 is the only variable associated with CKD development after controlling for confounders. Interestingly, LN and SLEDAI score no longer remain independent associated with CKD. The results, comparable to those in two previous studies (8, 37), which found that the adjusted mean nonrenal SLEDAI or WHO classification for LN

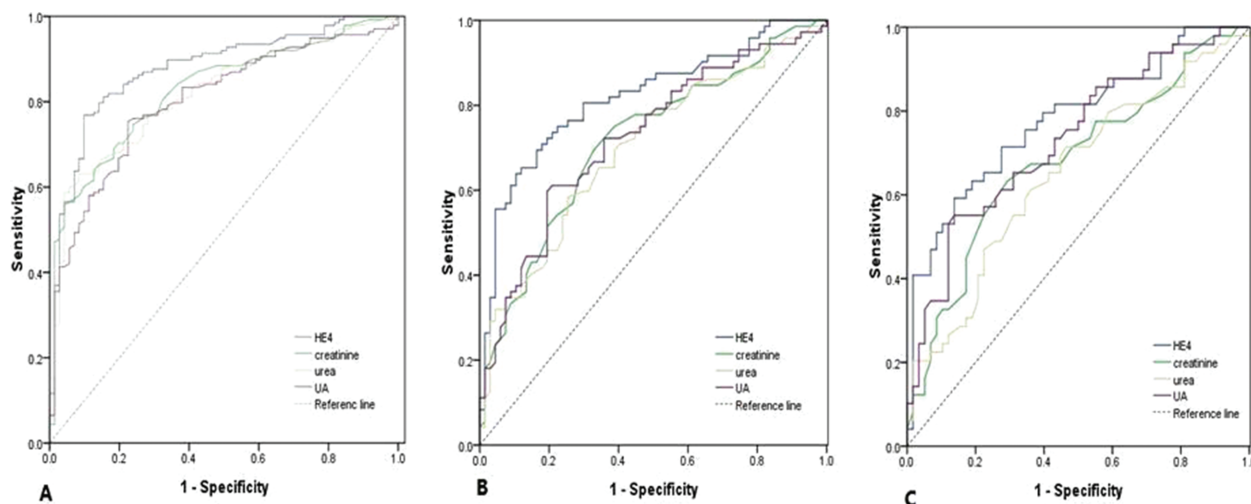


Fig. 2. ROC curves of HE4, creatinine, urea, and UA for the diagnosis of LN in SLE patients. (A) LN versus SLE without LN, (B) LN without CKD versus SLE without CKD, (C) LN with normal eGFR versus SLE with normal eGFR.

TABLE 3. Results for Measurement of Serum HE4, Creatinine, Urea, and UA in the Diagnosis of LN or CKD in SLE Patients

	AUC	95% CI	P	Cutoff	Sen (%)	Spe (%)	PPV (%)	NPV (%)	PLR	NLR	Accuracy (%)
LN versus SLE without LN											
HE4	0.878 ± 0.025	0.829–0.926	<0.001	150.1	76.8	91.1	94.6	67.0	8.629	0.255	81.8
Creatinine	0.827 ± 0.029	0.771–0.883	<0.001	65.5	74.6	76.1	85.8	60.7	3.121	0.334	75.1
Urea	0.818 ± 0.029	0.761–0.875	<0.001	7.75	60.1	94.4	95.4	54.9	10.732	0.423	71.8
UA	0.803 ± 0.031	0.742–0.863	<0.001	326.5	75.4	77.5	86.7	61.8	3.351	0.317	76.1
LN without CKD versus SLE without CKD											
HE4	0.818 ± 0.036	0.747–0.889	<0.001	113.1	63.9	89.6	86.8	69.8	6.144	0.403	76.3
Creatinine	0.716 ± 0.043	0.630–0.801	0.016	58.5	69.4	67.2	69.4	67.2	2.116	0.455	68.3
Urea	0.700 ± 0.044	0.614–0.787	<0.001	5.55	58.3	74.6	71.2	62.5	2.295	0.559	66.2
UA	0.724 ± 0.043	0.640–0.808	<0.001	326.5	59.7	70.6	68.3	61.8	2.031	0.571	64.7
LN with normal eGFR versus SLE with normal eGFR											
HE4	0.778 ± 0.045	0.689–0.867	<0.001	89.3	63.3	81.0	80.4	67.8	3.332	0.453	72.0
Creatinine	0.681 ± 0.053	0.578–0.784	0.001	57.5	63.3	70.7	72.6	64.8	2.160	0.519	67.3
Urea	0.649 ± 0.054	0.544–0.754	0.008	4.95	61.2	63.8	66.0	57.4	1.691	0.608	61.7
UA	0.733 ± 0.049	0.638–0.828	<0.001	231.0	85.7	44.8	64.9	73.3	1.553	0.319	67.3
CKD versus SLE without CKD											
HE4	0.948 ± 0.013	0.922–0.975	<0.001	233.9	92.9	93.5	89.0	96.3	14.292	0.076	93.3
Urea	0.920 ± 0.022	0.877–0.963	<0.001	10.25	81.4	89.9	80.3	90.6	8.059	0.207	87.1
UA	0.857 ± 0.030	0.798–0.916	<0.001	326.5	81.4	79.9	67.1	89.5	4.050	0.233	80.4
CKD4-5 versus SLE without CKD4-5											
HE4	0.959 ± 0.013	0.934–0.984	<0.001	390.3	97.1	84.5	55.7	99.3	6.265	0.034	86.6
Urea	0.944 ± 0.020	0.904–0.985	<0.001	12.35	91.4	86.2	57.1	98.0	6.623	0.100	87.1
UA	0.847 ± 0.036	0.776–0.917	<0.001	483.5	74.3	85.1	50.0	94.3	4.987	0.302	83.3
CKD in LN versus LN without CKD											
HE4	0.915 ± 0.023	0.870–0.960	<0.001	380.14	81.8	88.9	87.1	84.2	7.369	0.205	85.5
Urea	0.907 ± 0.025	0.858–0.956	<0.001	10.25	84.8	83.3	82.4	85.7	5.078	0.180	84.1
UA	0.813 ± 0.037	0.740–0.887	<0.001	479.5	66.7	86.1	81.5	73.8	4.799	0.387	76.8

Note: The unit of Cutoff was picomolar, millimolar per liter, and micromolar per liter for HE4, urea, and UA, respectively.

AUC, area under curve; UA, uric acid; LN, lupus nephritis; SLE, systemic lupus erythematosus; CKD, chronic kidney disease; Sen, sensitivity; Spe, specificity; PPV, positive predictive value; NPV, negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio.

by biopsy is not a critical risk factor in predicting CKD development in SLE patients, mean that CKD development may be independent of LN and SLE activity. Prompt identification for CKD is necessary in SLE patients, with or without LN. We found, in current study, serum HE4 is a good biomarker for identifying CKD in SLE patients. It can also be well used to identify ESRD or pre-ESRD (CKD4-5).

Although HE4 has been approved by U.S. Food and Drug Administration for monitoring recurrence and

progression of ovarian cancer, its clinical value has been frequently challenged by emerging studies that found significant increment of serum HE4 in CKD, renal failure, and heart failure (20–23). Our study adds additional evidence that serum HE4 could be a sensitive and specific biomarker for diagnosis of LN and CKD in SLE patients. Despite little known about the reasons for close association between HE4 and LN or CKD development in SLE, the facts that HE4 might have immunomodulatory properties and that it can be expressed in kidney could provide

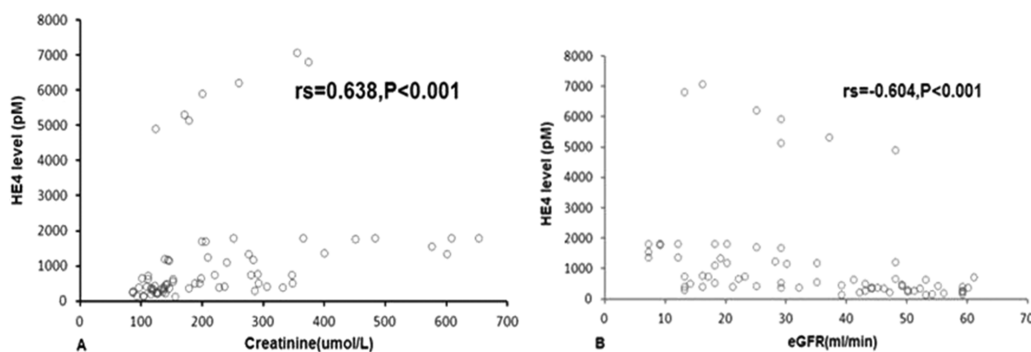


Fig. 3. Scatterplots showing the correlation between HE4 and creatinine(A) and eGFR(B).

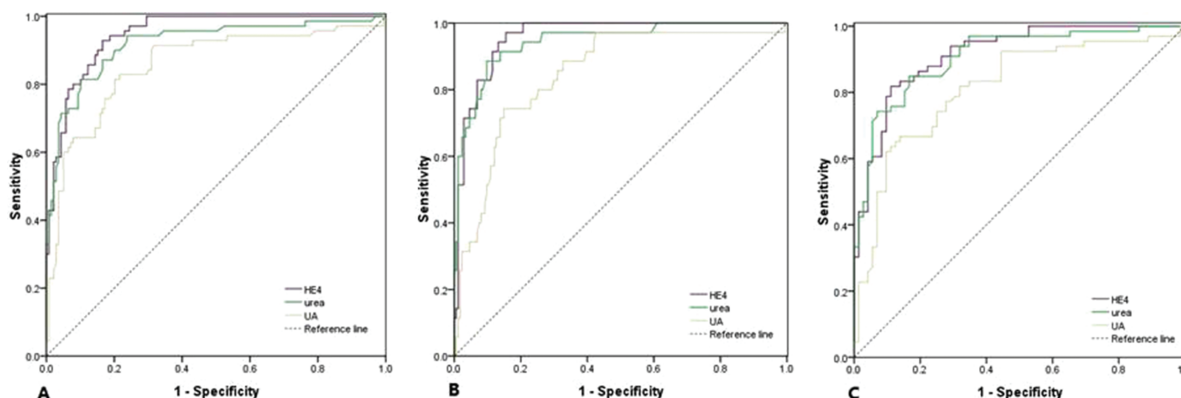


Fig. 4. ROC curves of HE4, urea, and UA for the diagnosis of CKD in SLE patients. (A) CKD versus SLE without CKD, (B) CKD4-5 versus SLE without CKD4-5, (C) LN with CKD versus LN without CKD.

some explanations (38, 39), since both LN and CKD are caused by systemic inflammation in SLE.

Although the precise mechanisms of HE4 elevation remain unknown, the renal dysfunction, which leads to the diminution of HE4 clearance, may be the main origin of HE4 elevation, because both the previous study (21) and our data indicated that the HE4 level is also significantly elevated in renal dysfunction by various risk factors rather than SLE. Furthermore, the elevated HE4 could contribute to CKD fibrosis, since a previous study reported that HE4 is a fibroblast-derived mediator of fibrosis and administration of HE4-neutralizing antibodies inhibited renal fibrosis (40). Therefore, HE4 elevation and renal dysfunction might be an interactive loop.

Some limitations should be addressed. First, our single-center study included relatively small size of population, which might bias the relationship between HE4 and LN or CKD in SLE patients. Therefore, the results from our data should be interpreted with caution and be further confirmed in a larger size of studies. Second, this is a cross-sectional study not designed to determine whether baseline or serial serum HE4 level can predict LN or CKD onset and progression. Also, this study cannot confirm a causative relationship between HE4 and LN or CKD. Future prospective studies are needed to establish the causation.

In summary, our study showed significantly increased serum HE4 level in SLE patients for the first time. In the clinical practice, use of different cutoff values of HE4 excellently discriminates between SLE patients with LN and CKD, confirming the important role of HE4 as a biomarker for the early diagnosis of LN and CKD in SLE.

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