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Serum human epididymis protein 4 is a predictor for developing nephritis in patients with systemic lupus erythematosus: A prospective cohort study

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ABSTRACT

Background: It has been demonstrated that serum human epididymis protein 4 (HE4) is a useful biomarker for differentiating lupus nephritis (LN) from systemic lupus erythematosus (SLE). However, it remains unclear whether HE4 can be used to predict the development of LN.

Methods: A total of 74 SLE patients without LN were recruited between August 2008 and September 2013. Serum HE4 concentrations were measured by enzyme-linked immunosorbent assay. These patients were followed up from the date of SLE diagnosis to LN development or the end of the study. The receiver operating characteristics (ROC) curve was drawn to assess the predictive value of HE4 for the incidence of LN. In addition, Kaplan–Meier and Cox regression analyses were used to determine the prognostic factors for the incidence of LN.

Results: Serum HE4 levels significantly increased in patients who are positive for anti-dsDNA antibody, low C3 and the incidence LN ($P < 0.05$), and these were closely correlated with age, erythrocyte sedimentation rate (ESR) and the SLE disease activity index (SLEDAI) ($P < 0.05$). During the follow-up, 44 patients developed LN. The ROC curve revealed that for HE4 levels, the predictive performance for LN with 64.8 pM as an optimal cutoff yielded an AUC of 0.714, with a 95% confidence interval of 0.597–0.831, and a sensitivity and specificity of 81.8% and 53.3%, respectively. The Kaplan-Meier analysis revealed that LN occurred in 72% of high-HE4 patients and 33.3% of low-HE4 patients ($P = 0.036$). The univariate analysis revealed that anti-dsDNA antibody, low C3, SLEDAI and HE4 were significantly associated with the incidence of LN ($P < 0.05$). Multivariate Cox regression revealed that only SLEDAI and HE4 were independently associated with the incidence of LN.

Conclusion: Elevated serum HE4 is significantly associated with a higher risk of incidence for LN, and may be a useful predictor for developing LN.

1. Introduction

Systemic lupus erythematosus (SLE) is a chronic and progressive autoimmune disease that involves multiple organs. Lupus nephritis (LN) is one of the most serious organs affected, which occurs in 50–70% of SLE patients [1]. LN is the major cause of morbidity and mortality in SLE patients [2], and the long-term survival of these patients can be improved with early diagnosis and prompt treatment for LN [3]. Therefore, it is of great importance to early detect LN. However, the insidious onset and fluctuating nature of LN make early identification and follow-up very difficult. In this sense, uncovering a biomarker that heralds the development of LN would be necessary for preventing or treating renal injury, constituting an unmet need in SLE patients.

Human epididymis protein 4 (HE4) was first identified and characterized as a human epididymis-specific protein in 1991 [4], and has been widely and routinely used as a tumor marker, particularly for ovarian cancer [5–8]. However, studies have shown that elevated serum HE4 can also occur in patients with chronic kidney disease, renal fibrosis, renal failure and heart failure [9–14]. Furthermore, the investigators previously found that serum HE4 is valuable for the differential diagnosis of LN in SLE patients [15]. Meanwhile, serum HE4 levels also increase in SLE patients without LN. It remains unclear whether serum HE4 can be used as an independent predictor for incident LN. Hence, this prospective study was conducted to address this question.

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2. Materials and methods

2.1. Patients

A total of 74 patients, who were newly diagnosed with SLE, which was defined as fulfilling at least four of the 1997 revised American College of Rheumatology (ACR) classification criteria for SLE [16], were recruited from Changzheng Hospital between August 2008 and September 2013. None of these patients had documented renal involvement or received any kidney-directed treatment. Peripheral venous blood was drawn at baseline, and sent for routine test analysis, including complement C3 and C4, anti-double strand DNA (dsDNA), -Sm, -SSA, -SSB and -URNP antibodies, erythrocyte sedimentation rate (ESR) and hypersensitive C-reactive protein (hsCRP), for assessing SLE activity. Sera were immediately prepared by centrifugation of venous blood, and processed with cryopreservation (−80 °C) for determining HE4 levels. The SLE disease activity index (SLEDAI) score [17] was calculated for each patient prior to starting the lupus treatment. Based on physical and radiological examinations and other examinations of the patient, ovarian disorders, endometrial cancer, lung cancer, or other tumors were not found at baseline. The patients subsequently visited Changzheng Hospital once or for various times per year, and were monitored for the development of LN without loss of follow-up. LN was pathologically confirmed by kidney biopsy, which was prompted when unexplained hematuria, proteinuria, or a decline in renal function occurred. These patients were followed up from the date of SLE diagnosis to LN development or the end of the present study (February 2018). The present study was approved by the Ethics Committee of the local institution, and an informed consent was obtained from each patient.

2.2. Measurement of serum HE4

All serum HE4 concentrations were measured in duplicate by enzyme-linked immunosorbent assay (ELISA; Fujirebio Diagnostics, Sweden), with a range of 15–900 pM. The inter- and intra-assay variations were < 8% and < 4%, respectively. Samples with HE4 concentrations of > 900 pM were measured again after dilution using an HE4 calibrator by 1/10, in accordance with the instructions.

2.3. Statistical analysis

SPSS 17.0 statistical software was used to analyze the data. Since all continuous variables, except for age, exhibited a skewed distribution, medians with interquartile ranges (IQRs) were used to describe the levels. Differences in HE4 levels between groups were compared using Mann–Whitney test. Spearman's correlation coefficient (rs) was used to evaluate the correlation between HE4 levels and other continuous variables. The receiver operating characteristic (ROC) curve was generated to evaluate the sensitivity, specificity and areas under the ROC curve (AUC) with the 95% confidence interval (CI). The optimal cutoff value for predicting the incidence of LN was identified by calculating the Youden index. The survival curves were drawn using Kaplan–Meier analysis, and compared by log rank test. Univariate and multivariate Cox regression analyses were used to determine the independent prognostic factors for LN development. It is noteworthy that in accordance with a previous study [18], log HE4 concentration was used as a risk factor in the Cox regression model. A change per unit in log-transformed HE4 concentration was equivalent to a 2.7-fold increase in HE4 concentration. A P-value < 0.05 was considered statistically significant.

3. Results

3.1. Baseline characteristics of SLE patients

Among the 74 SLE patients, 64 (86.5%) patients were female. The mean age (with standard deviation, SD in parentheses) of these patients was 40 (16) years old (range: 18–75 years old). Anti-dsDNA, -Sm, -SSA, -SSB and -URNP antibodies, and low C3 and C4 levels were detected in 22 (29.7%), 21 (28.4%), 51 (68.9%), 17 (23.0%), 28 (37.8%), 51(68.9%) and 44 (59.5%) SLE patients, respectively. During the follow-up, 49 (66.2%) and 15 (20.3%) patients took prednisolone and cyclophosphamide, respectively. All patients had good medication adherence during the follow-up period. LN developed in 44 patients (59.5%).

Table 1
The association between baseline HE4 with clinical and laboratory characteristics (categorical variables) in SLE patients.

	Patients (n)	HE4 (pM)	
		Median (IQR)	*P* value
Sex			
Male	10	71.7 (54.1–197.3)	
Female	64	68.5 (62.1–100.2)	0.728
Prednisolone			
Yes	49	70.7 (64.6–148.8)	0.226
No	25	66.9 (50.7–102.5)	
Cyclophosphamide			
Yes	15	74.7 (65.0–120.9)	
No	59	67.9 (57.9–95.9)	0.506
Anti-dsDNA			
Yes	22	98.6 (66.3–199.8)	
No	52	67.6 (56.6–86.2)	0.019
Anti-Sm			
Yes	21	67.8 (53.5–95.9)	
No	53	69.4 (64.4–143.6)	0.359
Anti-SSA			
Yes	51	67.9 (56.3–101.6)	
No	23	74.6 (64.4–131.3)	0.201
Anti-SSB			
Yes	17	66.0 (50.9–94.9)	
No	57	71.3 (64.6–148.8)	0.052
Anti-U1RNP			
Yes	28	73.3 (62.7–124.3)	
No	46	68.0 (55.2–135.8)	0.533
Low C3			
Yes	51	71.9 (62.3–179.4)	
No	23	66.4 (50.7–74.6)	0.029
Low C4			
Yes	60	69.9 (62.1–116.5)	
No	14	67.7 (55.0–97.5)	0.548
LN			
Yes	44	72.4 (66.2–192.9)	
No	30	64.4 (51.4–75.9)	0.002

SLE, systemic lupus erythematosus; HE4, human epididymis protein 4; IQR, interquartile range.

3.2. The relationship between HE4 levels and baseline clinical and laboratory variables

The associations between serum HE4 levels and categorized and continuous variables are presented in Tables 1 and 2, respectively. Serum HE4 levels significantly increased in patients with positive anti-dsDNA antibody (98.6 pM *vs*. 67.6 pM, $P = 0.019$), low C3 (71.9 pM *vs*. 66.4 pM, $P = 0.029$), and incident LN (72.4 pM *vs*. 64.4 pM, $P = 0.002$), when compared with patients without these. Furthermore, serum HE4 levels exhibited a close correlation with age ($r = 0.266$, $P = 0.022$), ESR ($r = 0.376$, $P = 0.001$) and SLEDAI ($r = 0.289$, $P = 0.013$). However, no significant association existed between serum HE4 levels and other variables.

3.3. The predictive value of HE4 for incident LN in SLE patients

The median follow-up duration was 5.7 years (range: 1.0–9.5 years). During the follow-up, 44 SLE patients developed LN. The ROC curve