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# Serum human epididymis secretory protein 4 as a potential biomarker of renal fibrosis in kidney transplantation recipients

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ABSTRACT

*Background:* Renal fibrosis remains an important cause of kidney allograft failure. The objective of this study was to evaluate the performance of serum human epididymis secretory protein 4 (HE4) as a biomarker for renal fibrosis in kidney transplant recipients.

*Methods:* A total of 103 kidney transplantation patients were enrolled in this study, and serum HE4 concentrations were detected using the chemiluminescent microparticle immunoassay. Renal biopsy was carried out, and histological findings were assessed by immunohistochemistry.

*Results:* Median serum HE4 concentrations were significantly increased in kidney transplant recipients (186.2 pmol/l, interquartile range [IQR] 125.6–300.2) compared with control subjects (34.3 pmol/l, IQR 30.4–42.3, $p < 0.0001$). Meanwhile, serum HE4 concentrations were significantly increased along with disease severity ($p < 0.0001$). In addition, we found serum HE4 concentrations to be strongly correlated with the severity of fibrosis (IF/TA 0, 1, 2, and 3: 114.3, 179.0, 197.8, and 467.8 pmol/l, respectively; $p < 0.0001$) and serum HE4 concentrations significantly correlated with HE4 tissue expression concentrations in renal biopsy.

*Conclusions:* Serum HE4 was increased in kidney transplant recipients with decreased kidney function and renal fibrosis and was correlated with the severity of the disease, suggesting that HE4 has the potential to be used as a novel clinical biomarker for evaluating kidney function and predicting renal fibrosis in kidney transplant recipients.

## 1. Introduction

Chronic kidney disease is extremely widespread in developing countries [1]. As the world's largest developing country, China is confronting a serious public health problem caused by chronic kidney disease, which is affecting approximately 10.8% of the adult population [2]. Advanced chronic kidney disease can be accompanied by serious complications, increased risk for cardiovascular diseases and death, and the need for kidney transplant therapy [3]. Kidney transplantation is recognized as the most optimal treatment for patients with end-stage renal disease, as it has been shown to improve quality of life as well as survival rates [4]. However, chronic renal allograft failure has a serious influence on the effective life of the allograft and is characterized by tubular atrophy, arteriolar hyalinosis, interstitial fibrosis, and glomerulosclerosis [5,6]. Renal fibrosis, a dynamic system that involves extracellular matrix components as well as renal and other infiltrating cell types, progresses well before obvious clinical dysfunction results in the eventual endpoint of all progressive kidney diseases [7]. Previous studies have suggested that progressive fibrosis is determined by the cumulative damage from a series of attacks, including cellular rejection, antibody-mediated rejection, ischemia reperfusion injury, hypertension, and calcineurin nephrotoxicity [5,8]. Generally, its recognition is relatively delayed and usually occurs when pathological biopsies for cause are performed to investigate deteriorating graft function and no

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obvious etiology is identified. Thus, novel biomarkers may have the potential to improve risk assessment in patients with renal fibrosis.

Serum sample analysis is a convenient, noninvasive technique, and it is not subject to operator diversity, for example, imaging analysis. Thus, substantial efforts have been undertaken to search for novel serum biomarkers for disease diagnosis. Human epididymis secretory protein 4 (HE4), encoded by the Whey-Acidic Four-Disulfide Core domain protein 2 (WFDC2) gene, is located on chromosome 20q12-13.1 [9]. As a secreted tumor biomarker, HE4 is identified as a consistent biomarker in epithelial ovarian cancer, endometrial carcinomas, and lung malignancies [10–15]. Also, it is identified as a potential biomarker for detection of renal fibrosis, cystic fibrosis, and a potential target to inhibit fibrosis [16–18]. However, the concentrations of HE4 in kidney transplant recipients are still unknown.

## 2. Materials and methods

### 2.1. Subjects

A total of 103 Chinese renal biopsy recipients who underwent renal transplantation at the First Affiliated Hospital of Sun Yat-sen University between January 2005 and December 2015 were selected. The renal biopsy was performed between February 2016 and June 2016. No recipients had a history of smoking, malignancy, acute kidney injury, immunodeficiency, chronic lung disease, or liver disease. The control group consisted of 127 age-matched healthy subjects.

This study was reviewed and approved by the Institutional Review Board of the First Affiliated Hospital of Sun Yat-sen University according to the guidelines of Helsinki conventions. All subjects enrolled in this study gave written informed consent for participation in the study and for the use of their samples.

### 2.2. Biopsies and tissue processing

Renal biopsies were performed by an automated biopsy gun with an 18-gauge needle, according to ultrasound guidance. Pathologic samples were analyzed in the Department of Pathology of the First Affiliated Hospital of Sun Yat-sen University according to standard hematoxylin and eosin (HE) staining procedures to assess glomerular, renal tubular, and interstitial status. The slides were reviewed and scored independently according to the Banff classification by two qualified pathologists who were blinded to the specimen's clinical characteristics [19]. Briefly, the grade of fibrosis was based on interstitial fibrosis/tubular atrophy (IF/TA), according to the percentage of cortical parenchymal involved, as follows: IF/TA 0, interstitial fibrosis $\leq$5% cortical area; IF/TA 1, 6% to 25%; IF/TA 2, 26% to 50%; IF/TA 3, > 50%.

### 2.3. Immunohistochemistry

Immunohistochemistry (IHC) staining of formalin-fixed, paraffin-embedded samples was processed and quantified as previously described [20]. Briefly, 5-μm slides were deparaffinized, rehydrated through a series of descending graded alcohols, and treated with 0.01 mol/l citrate buffer (pH 6.0) at 90 °C for 40 min for antigen retrieval. Following a blocking step, the slides were incubated with antibody against HE4 (1200; Sigma) for 30 min at room temperature and then washed, followed by biotinylated secondary antibody (Vector Labs). Signal detection was performed using an avidin-biotin complex immunoperoxidase system (Vector) with 0.03% diaminobenzidine as a chromagen and counterstained with hematoxylin.

### 2.4. Clinical variables and serum HE4 tests

Clinical information and laboratory data were collected from electronic databases and patient medical records. For laboratory tests, venous blood samples were taken in the morning after an overnight fast of at least 8 h. All blood samples were centrifuged immediately and tested within 2 h in the Department of Clinical Laboratory of the First Affiliated Hospital of Sun Yat-sen University. HE4 was tested by Architect HE4 assay (Abbott Diagnostics) according to the manufacturer's instructions.

### 2.5. Statistical analysis

All statistical analyses were performed using SPSS ver 18.0 software. Data with normal distribution were expressed as mean ± SD and were analyzed by Student's independent *t*-test analysis. Clinical variables and laboratory data with nonnormal distribution were analyzed by Mann-Whitney *U* test. The differences between categorical variables were analyzed by chi-square test. The correlation analysis was calculated using Spearman's correlation. The discriminative power of HE4 was evaluated by receiver-operating characteristics (ROC) curve analysis, and the cutoff value was calculated using the score closest to the value with both maximum sensitivity and specificity. In all cases, a $p < 0.05$ was considered to be statistically significant, and all tests were performed as 2-tailed tests.

## 3. Results

### 3.1. Study population characteristics

Kidney transplant recipients and control participants were matched for age and sex (Table 1). In both the kidney transplant recipients and the control group, frequency distributions of serum HE4 concentrations presented as nonnormal distributions (Fig. 1A and B), which was verified by Kolmogorov-Smirnov test. Compared with the control subjects, the median HE4 concentrations in kidney transplant recipients were obviously increased (34.3 pmol/l, interquartile range [IQR] 30.4–42.3 vs. 186.2 pmol/l, IQR 125.6–300.2, $p < 0.0001$; Fig. 1C, Table 1). Consistent with the HE4 concentrations, serum creatinine (Scr) concentrations were significantly increased in patients with kidney transplant compared with control subjects (median 170.0 μmol/l, IQR 129.5–236.5 vs. 64.8 μmol/l, IQR 60.0–72.3, $p < 0.0001$; Table 1). Median HE4 concentrations were significantly increased in male kidney transplant recipients (174.6 pmol/l, IQR 115.6–272.6) and female kidney transplant recipients (242.0 pmol/l, IQR 160.0–317.3) compared with control subjects (36.0 pmol/l, IQR 30.9–42.9, $p < 0.0001$; Fig. 1D). However, no statistically significantly difference in serum HE4 concentration was observed between the male and the female patients ($p = 0.0596$; Fig. 1D).

Baseline parameters of the total cohort of kidney transplant recipients and as stratified below and above the HE4 median are displayed in Table 2. Subjects with serum HE4 concentrations above the median were older, had higher systolic blood pressure, and a longer duration of time since kidney transplantation ($p < 0.05$). In addition, hemoglobin, phosphorus, and urea concentrations were higher in the group that had HE4 concentrations above the median ($p < 0.05$). Moreover, serum creatinine concentrations were obviously higher and

**Table 1**
Baseline Clinical characteristics of patients with kidney transplantation and control subjects.

| Characteristic | Control (n = 127) | Recipient (n = 103) | *P* Value |
|---|---|---|---|
| Age (y) | 41.2 ± 11.7 | 38.8 ± 11.7 | NS |
| Gender (M/F), n/n | 91/36 | 78/25 | – |
| Scr (μmol/l) | 64.8 (60.0–72.3) | 170.0 (129.5–236.5) | < 0.0001 |
| HE4 (pmol/l) | 34.3 (30.4–42.3) | 186.2 (125.6–300.2) | < 0.0001 |

Data are expressed as means ± SD for normally distributed continuous variables, as median and interquartile range for continuous variables that are not normally distributed. Scr: serum creatine, HE4: human epididymis secretory protein 4.