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Human epididymis protein 4 concentration is not associated with liver fibrosis and cirrhosis in a case control study

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ABSTRACT

Background: Human epididymis protein 4 (HE4) is an emerging fibrotic biomarker which has been studied in chronic kidney disease cohorts. However, it is unclear if the serum level of HE4 may be altered in patients with liver fibrosis and cirrhosis.

Methods: we assessed serum HE4 concentrations in patients (n = 366) with chronic liver diseases (CLD) and compared to matched healthy controls (n = 366). Liver stiffness measurement (LSM) by transient elastography (TE, FibroScan) was also performed on all patients. Liver biopsy was performed on 34 of 366 subjects. Moreover, we analysed a subgroup of patients with confirmed cirrhosis to validate the correlation between HE4 and the severity of cirrhosis. Child-Pugh (CP) score was evaluated in this subgroup.

Results: No statistically significant differences were observed in the median HE4 level between patients with fibrosis and cirrhosis and controls (median: 56.2 vs. 55 pmol/L, p = .562). Neither were any significant differences found among different groups with Child-Pugh Classes A, B and C (median: 56.9, 58.3 and 52.1 pmol/L, respectively; p = .842). Correlation analysis did not show a significant correlation between HE4 and degree of liver fibrosis according to LSM values or histological assessment (r = 0.159, p = .239; r = 0.045, p = .788).

Conclusions: Serum HE4 level does not appear to be associated with fibrotic and cirrhotic liver, suggesting that HE4 may not serve as a valuable clinical biomarker for liver fibrosis and cirrhosis.

1. Introduction

Human epididymis protein 4 (HE4, also termed WFDC2) is a secretory protein, which was originally identified as a transcript exclusively expressed in the human epididymis [1, 2]. After the initial studies, HE4 was also reported expressed in some ovarian malignancy women's blood [3] and shown to be a serum biomarker for diagnosis of ovarian cancer [4–7]. Meanwhile, biomarker potential of HE4 has been investigated in other diseases, such as chronic kidney disease (CKD) and heart failure (HF) [8–11]. Nagy et al. [8] described increased HE4 levels in women with CKD and another study [9] including both female and male found that HE4 was a predictor of advanced renal fibrosis in all CKD patients. In a recently published study by Valerie LeBleu and colleagues [9] similar outcomes were observed in both genetic mouse models of renal disease and patients with fibrotic kidneys. In addition, the researchers also suggested that HE4 might serve as a new target for treatment of renal fibrosis. More recently, circulating HE4 levels has been found to be correlated with HF severity and strongly predictive of HF outcome [11, 12]. Furthermore, the authors showed that HE4 was strongly associated with HF fibrosis biomarkers such as galectin-3 [13].

Liver fibrosis results from chronic liver disease of all etiologies. It is the forming scar and chronic pathological remodeling of the liver, in which the normal hepatic tissue architecture is progressively replaced by excessive collagenous extracellular matrix (ECM). ECM proteins are secreted by myofibroblasts, which are the source of the fiber scar in the kidneys, lungs, and liver [14, 15]. Myofibroblasts are absent from normal tissues and imbedded in the fibrous scar of the liver [16–18]. Evidence from animal models and clinical trials indicates that there is a close association between the regression of liver fibrosis and the disappearance of these myofibroblasts [19]. However, the functional contribution of myofibroblasts in fibrosis has not been thoroughly elucidated. Recently, LeBleu et al. [9] revealed that myofibroblasts could robustly express HE4 protein, which suppressed Prss35 and Prss23 serine protease activity and specifically inhibited their capacity to degrade type I collagen.

Given the data, HE4 seems to be a mediator of fibrosis in patients

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with different fibrotic diseases. The aim of our study was therefore to evaluate whether or not serum HE4 could constitute a potential new liver fibrosis biomarker.

2. Materials and methods

2.1. Patients

From January 2016 to January 2017, a total of 366 consecutive patients with chronic liver disease at the Shanxi DaYi Hospital in Taiyuan, China, were prospectively enrolled in the study. Exclusion criteria were as follows: was pregnant/puerperium, had a history of malignancy, had an estimated glomerular filtration rate (eGFR) $< 60\,mL\,min^{-1}\ 1.73\,m^{-2}$ and had no valid transient elastography measurements available. At the same period, 366 control subjects were recruited from health check-up participants who visited Shanxi DaYi Hospital and were closely matched for age, sex, and body mass index (BMI). Controls were apparently healthy subjects, without overt cause of liver disease and with normal liver enzymes. The study protocol conformed to the Declaration of Helsinki and was approved by the local medical ethical committee. Written informed consent to participate in the study was obtained from each subject.

3. Methods

3.1. Data collection and laboratory tests

Participant demographics and clinical data were recorded, including age, sex, body mass index, etiology of chronic liver disease and blood pressure (SBP and DBP, respectively). Blood samples were drawn between 8 AM and 10 AM after an overnight fast of at least 8 h. Routine laboratory measurements were performed within 2 h in the clinical laboratory department of our hospital. For HE4 assays, serum samples were separated and stored at −70 °C before analysis. The 4v-MDRD formula was used to calculate eGFR ($mL\,min^{-1}/1.73\,m^2$) [20].

3.2. Measurement of HE4

Human serum HE4 levels were tested with the use of an enzyme immunometric assay (EIA; Fujirebio Diagnostics, Gothenburg, Sweden). Performance characteristics of HE4 EIA assay according to the manufacturer's instructions were as follows: The HE4 assay precision is ≤15%total CV; This kit has a detection range between 15 and 900 pmol/L; The functional sensitivity of the HE4 EIA assay is≤25 pmol/L. The functional sensitivity is expressed as the concentration of an analyte at which the CV is 20%; The HE4 EIA assay mean recovery is 100 ± 15%; the mean dilution linearity is 100 ± 15% and the mean assay specificity is 100 ± 15%.

Both calibrators and controls were performed in duplicate for each assay. The lower control has a range of expected value between 33 and 62 pmol/L and the corresponding value of upper control was 311–466 pmol/L.

3.3. Transient elastography

Liver stiffness measurement (LSM) was performed by transient elastography (Fibroscan, Echosense, France), which is a useful non-invasive tool to diagnose grade of fibrosis/cirrhosis [21, 22]. The examination was performed in a supine position with the right arm in maximal abduction. At least ten measurements were performed for each patient, and the median value was then taken into account. Only examinations with a success rate of at least 60% and an IQR/M ratio of 30% were classified as valid for statistical analysis. If the criteria described above were not fulfilled, the test was considered as invalid. The results were expressed in kilopascal (kPa). LSM categorized fibrosis according to four levels of severity: S1 = none to mild (METAVIR scoring system F0-1, < 7.1 kPa), S2 = moderate (F2, > 7.1 to < 9.6 kPa), S3 = severe (F3, ≥9.6 to < 12.5 kPa) and S4 = cirrhosis (F4, ≥12.5 kPa) [22–25].

3.4. Liver histology

In this study, liver biopsies were performed on 34 of 366 subjects. Ultrasound-guided percutaneous liver biopsy was performed using the Menghini technique with a 16-G Hepafix needle (Braun Medical, Melsungen, Germany). Liver histology was interpreted by the same experienced pathologist blinded to the clinical data. Fibrosis was evaluated according to the METAVIR scoring system [26]: F0 = no fibrosis; F1 = portal fibrosis without septa; F2 = portal fibrosis and few septa; F3 = numerous septa without cirrhosis; and F4 = cirrhosis.

3.5. Subgroup definition

We analysed 47 patients with cirrhosis to identify the HE4 levels in cirrhosis cohorts. Cirrhosis was defined by the presence at least two of the following five criteria [27]: (1) radiographic (ultrasound, computed tomography, and magnetic resonance imaging) evidence of liver nodularity; (2) radiographic evidence of portal hypertension; (3) platelet count $< 120 \times 10^9$/L; (4) endoscopic evidence of varices or portal hypertensive gastropathy; or (5) liver biopsy with METAVIR stage 4 in the past. Child-Pugh score including five variables (i.e., TBIL, ALB, PT, hepatic encephalopathy, and ascites) was calculated [28].

3.6. Statistical analyses

All continuous variables were reported as the mean ± standard deviation (SD) or median (interquartile range [IQR]). Student *t*-test was performed for group comparisons of normally distributed data and Mann–Whitney *U* test where data was non-normally distributed. The correlation between two variables was determined by Spearman's and Pearson's correlation analysis where appropriate. The diagnostic performance of serum HE4 for liver fibrosis was determined using ROC curves. Two-sided *p*-values < 0.05 were considered statistically significant. Data analyses were conducted using SPSS Version 18.0 (SPSS Inc., Chicago, IL, USA).

4. Results

4.1. Study population and baseline characteristics

Baseline characteristics of the study participants are presented in Table 1 according to the stage of liver fibrosis. A total of 366 patients were included in the study. These patients were predominantly men [*n* = 244 (66.9%)] with a mean age of 47.6 year-old. For the control group, mean age was 45.9 years, and 66.9% of controls were also male. Aetiologies of chronic liver diseases were: HBV (*n* = 142), HCV infection (*n* = 76), alcoholic liver disease (*n* = 88), autoimmune (*n* = 26), and other (*n* = 34). Fibrosis stage distribution according to FibroScan was as follows: 212 patients (57.9%) had no or mild fibrosis (S1), 65 patients (17.7%) had moderate fibrosis (S2), 45 patients (12.3%) had severe fibrosis (S3), and 44 patients (12.0%) had cirrhosis (S4).

4.2. Multivariable analysis

Multiple linear regression was performed in order to identify factors that were independently associated with log-transformed HE4 levels. For patients, total bilirubin was positively associated with HE4 (*p* = .014). No other factors were significant in this model (Table 2). For controls, there was no such associated factor by the same multivariable statistical test (data not shown).