Clinical Trial/Experimental Study

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Clinical significance of serum human epididymis protein 4 in liver fibrosis

An experimental study

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Abstract

Background: Human epididymis protein 4 (HE4) has been identified as marker for renal fibrosis. Present study aimed to investigate the clinical significance of serum HE4 in liver fibrosis.

Methods: Serum from 65 liver fibrosis patients, 68 hepatic patients without fibrosis, and 50 controls was collected respectively. Serum HE4 levels were measured by chemiluminescence immunoassay and compared among the groups. The relationships between serum HE4 levels and the clinical characteristics of liver fibrosis were also analyzed. A receiver operator characteristic curve was plotted to investigate the diagnostic efficacy of serum HE4 for liver fibrosis. Child–Pugh (C–P) score and liver fibrosis score were also evaluated. Data were analyzed by statistical software 13.0.

Results: Serum HE4 levels were significantly higher in liver fibrosis than that of controls [105.35 (82.64, 164.18) vs 46.2 (39.9, 58.9) pmol L^{-1}, $P = .00$] and hepatic patients without liver fibrosis [105.35 (82.64, 164.18) vs 51.00 (44.02, 65.65) pmol L^{-1}, $P < .01$]; Serum HE4 levels in liver fibrosis patients with C–P class C were significantly higher than those with C–P class A [143.75 (106.50, 186.08) vs 81.42 (69.73, 99.26) pmol L^{-1}, $P = .005$] and C–P class B [143.75 (106.50, 186.08) vs 113.10 (88.92, 169.50) pmol L^{-1}, $P = .01$]; the diagnostic sensitivity and specificity of serum HE4 levels for liver fibrosis detection were 87.5% and 81.1%, at a cutoff value of 69 pmol L^{-1}; Serum HE4 levels in alcoholic liver fibrosis were higher than that of liver fibrosis with hepatitis B virus infection [131.30 (100.67, 228.35) vs 89.46 (73.74, 116.45) pmol L^{-1}, $P < .01$].

Conclusion: Serum HE4 was closely correlated with C–P class and might be a potential marker for liver fibrosis.

Abbreviations: ALD = alcoholic liver disease, AUC = the area under the curve, C–P = Child–Pugh, HBV = hepatitis virus B, HCV = hepatitis virus C, HE4 = human epididymis protein 4, NASH = non-alcoholic steatohepatitis, ROC = non-alcoholic steatohepatitis .

Keywords: diagnostic efficacy, liver fibrosis, serum human epididymis protein 4

1. Introduction

Liver fibrosis is a common wound-healing response to chronic liver injuries, including viral infection and persistent alcoholic toxicity.[1] The worst clinical result of liver fibrosis is development of hepatocellular carcinoma, while most early stages of fibrosis remain asymptomatic. Although liver biopsy is a standard method for liver fibrosis diagnosis, some limitations such as invasiveness, sampling variability and risk of bleeding usually appear.[2] Therefore, it has poor acceptance, especially when repeated measures are required.

Non-invasive serum biomarkers are increasingly used for liver fibrosis detection. The existing biomarkers for fibrosis lacked enough specificity and sensitivity, especially, the biomarkers for early fibrosis are still lacked. Therefore, looking for liver fibrosis markers is urgently needed.

Human epididymis protein 4 (HE4, also named WFDC2) is a secreted protein, which is widely used as a marker for ovarian cancer.[3] However, recent studies indicated that HE4 played critical roles in fibrosis.[4–6] Zhang et al reported that hypoxia promoted extracellular matrix accumulation and renal fibrosis by increasing HE4 expression in tubular epithelial cells.[7] Serum HE4 as a marker for renal and lung fibrosis has been reported.[8,9] Notably, liver fibrosis has much in common with renal fibrosis, such as the structural components of the fibrotic extracellular matrix, growth factors, cytokines, chemokines, and proteases, as well as central signaling cascades implicated in fibrosis, which are nearly identical in these different tissues.[10] The present study aimed to investigate the clinical significance of serum HE4 in liver fibrosis.

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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2. Materials and methods

2.1. Subjects

Eligible patients were identified at the department of infectious Disease, the First Affiliated Hospital of Chongqing Medical University, China. From January to October 2019, serum was collected from 65 liver fibrosis patients (49 male, 16 female; age: 53.3 ± 6.2 years), 68 hepatic patients without liver fibrosis (25 patients with hepatitis virus B (HBV), 15 patients with hepatitis virus C (HCV), 17 patients with non-alcoholic steatohepatitis (NASH), 11 patients with acute liver injury) and 50 healthy volunteers (26 male, 24 female, age: 48.5 ± 4.3 years) as the control group. Patients were excluded as follows: history of cancer and estimated glomerular filtration rate < 60/mL/1.73m^2. All patients underwent liver biopsy and the pathological diagnosis was confirmed by histological examination. Fibrosis was identified according to the METAVIR scoring system[11]: F1, portal fibrosis without septa; F2, portal fibrosis and few septa; F3, numerous septa without fibrosis; F4, fibrosis. Child–Pugh (C–P) class was calculated using 5 variables according to reports.[12] All patients and healthy volunteers provided written consent to participate in the study and the protocol was approved by the Ethics Committee of the First Affiliated Hospital of Chongqing Medical University.

2.2. Sample collection

Venous blood (2.0 mL) was collected from each subject into tubes without anticoagulant. Serum was separated by centrifugation at 2000 g for 10 minutes, samples were then stored at − 80°C until use.

2.3. Serum HE4 measurements

Serum HE4 levels were measured by chemiluminescence immunoassay (E602, Roche Diagnostics, Germany). The intermediate precision for the HE4 assay was1.5% and the measuring range was from 15 to 1500 pmol L^{-1}.

2.4. Statistical analysis

Data were analyzed using the Mann–Whitney *U* test. Serum HE4 levels were summarized as median and quartile M (P25, P75). A receiver operating characteristic (ROC) curve was plotted to determine the area under the curve (AUC), sensitivity and specificity of serum HE4 levels. The relationship between serum HE4 and patient characteristics was investigated using Spearman analysis. Statistical analysis was performed using SPSS 13.0 software (SPSS, Inc; Chicago, IL, USA); $P < .05$ represented a statistically significant result.

3. Results

3.1. Clinical characteristics of subjects

A total of 65 (49 male and 16 female) patients with liver fibrosis were enrolled in the study. The clinical characteristics are summarized in Table 1. According to the C–P class, 16 patients (24.6%) were A, 27 patients (41.6%) B and 22 (33.8%) C. According to the METAVIR Scoring system, the liver fibrosis score was as follows: F1: 13patients (26.6%); F2: 15 patients (30.6%); F3: 10 patients (20.4%) and F4: 11 patients (22.4%). Hepatic patients without fibrosis comprised 25 with HBV, 15 with HCV, 17 with NASH and 11 with acute liver injury.

3.2. Serum HE4 levels in liver fibrosis patients

Serum HE4 levels in liver fibrosis patients, hepatic patients without liver fibrosis and healthy controls were 105.35 (82.64, 164.18) pmol L^{-1}, 51.00(44.02,65.65) pmol L^{-1} and 46.2 (39.9, 58.9) pmol L^{-1} respectively. Further analysis showed that serum HE4 levels were higher in liver fibrosis patients compared with healthy controls ($P = .00$) and hepatic patients without liver fibrosis ($P = .001$), but no significant differences were found between hepatic patients without liver fibrosis and healthy controls($P > .05$; Fig. 1).

3.3. Serum HE4 levels among different C–P classes and fibrosis scores

Serum HE4 levels in liver fibrosis patients with C–P class C were higher than that of C–Pclass A [143.75 (106.50, 186.08) vs 81.42 (69.73, 99.26) pmol L^{-1}, $P = .005$] and class B [143.75 (106.50, 186.08) vs 113.10 (88.92, 169.50) pmol L^{-1}, $P = .01$] (Fig. 2A); further analysis indicated that no significance was found in serum HE4 among patients with F1, F2, F3, and F4 [78.12 (65.22,98.73) pmol L^{-1} vs 90.00 (73.74,122.55) pmol L^{-1} vs 113.35 (91.40,122.10) pmol L^{-1} vs 200.41 (147.50,250.52) pmol L^{-1}, $P = .053$]. However, it is interesting that the higher of the serum HE4 levels, the worse fibrosis appeared; in addition, serum HE4 levels in the various groups were compared individually, results indicated that higher HE4 levels were found in F3 than that of F1 ($P = .01$), higher HE4 levels were found in F4 than that of F1 ($P < .01$), F2 ($P = .02$) or F3 ($P = .013$), as shown in Figure 2B. Further analysis indicated that serum HE4 levels in high scores (F3+F4) were higher that of low scores (F1+F2)

Table 1

Clinical characteristics of patients.

Characteristics	n(%)
Sex F/M	16/49 (24.6/75.4)
Age (yr)	53.3 ± 6.2
Severity grading of fibrosis	
Child–Pugh class	
A	16 (24.6)
B	27 (41.6)
C	22 (33.8)
METAVIR scoring system*	
F1	13 (26.6)
F2	15 (30.6)
F3	10 (20.4)
F4	11 (22.4)
Etiologies of hepatic patients	
Hepatic patients with fibrosis	
HBV infection	40 (61.5)
Severe alcoholics	25 (38.5)
Hepatic patients without fibrosis	
HBV	25 (36.8)
HCV	15 (22.1)
NASH	17 (25.0)
Acute liver injury	11 (16.1)

* some data missed.

HBV = hepatitis virus B, HCV = hepatitis virus C, NASH = non-alcoholic steatohepatitis.

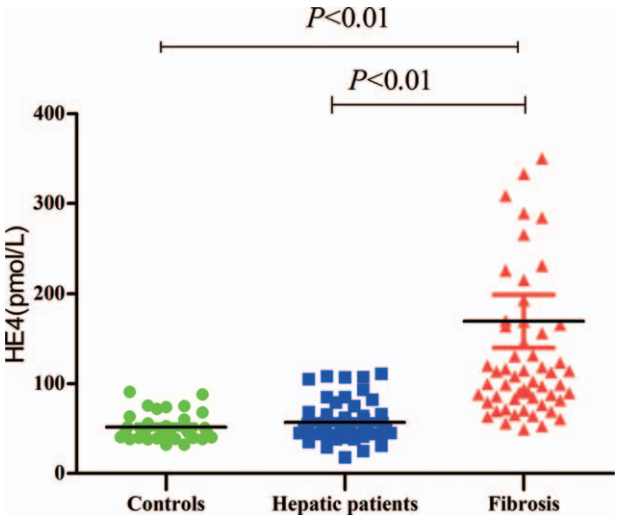

Figure 1. Serum human epididymis protein 4 levels among different groups.

[131.30 (102.6, 215.40) vs 86.55 (69.93, 101.86) pmol L^{-1}, $P = .004$, Fig. 2C].

3.4. Diagnostic efficacy of serum HE4 levels for liver fibrosis

The ROC curve of serum HE4 levels for fibrosis is shown in Figure 3 with an AUC of 0.921 (95%CI: 0.880–0.962). According to the rule of the maximum Yoden index, 69 pmol L^{-1} was set as the optimum cut-off value, with a sensitivity of 87.5% and specificity of 81.1%.

3.5. Relationships among different etiologies of hepatic patients

Serum HE4 levels in alcoholic liver disease (ALD) (fibrosis patients with ALD) were higher than that of chronic liver disease caused by HBV infection (fibrosis patients infected with HBV) [131.30 (100.67, 228.35) pmol L^{-1} vs 89.46 (73.74, 116.45) pmol L^{-1}, $P = .003$], as shown in Figure 4. Further analysis indicated that no significant association was found between

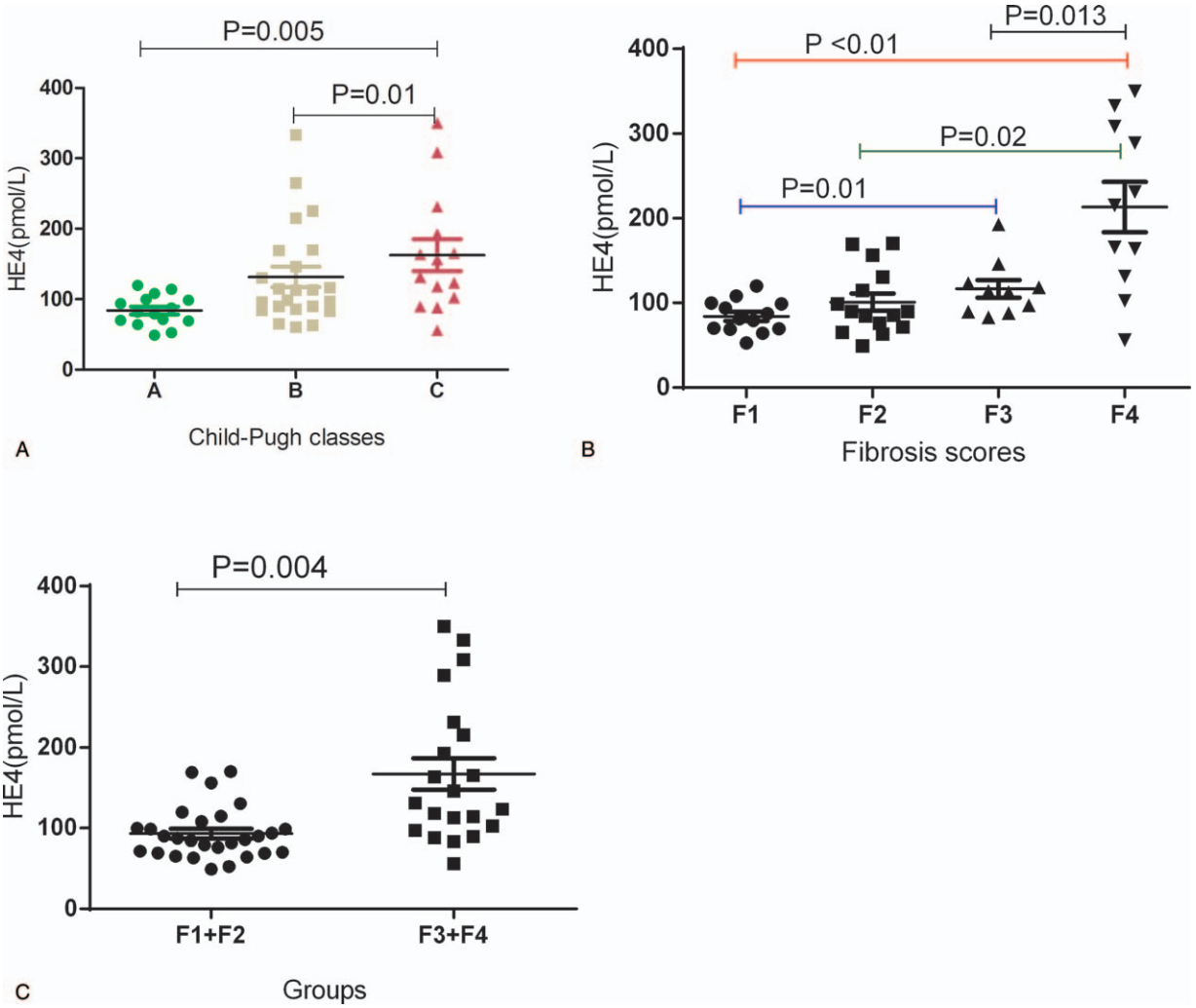

Figure 2. Serum human epididymis protein 4 levels among different Child–Pugh scores or fibrosis scores.

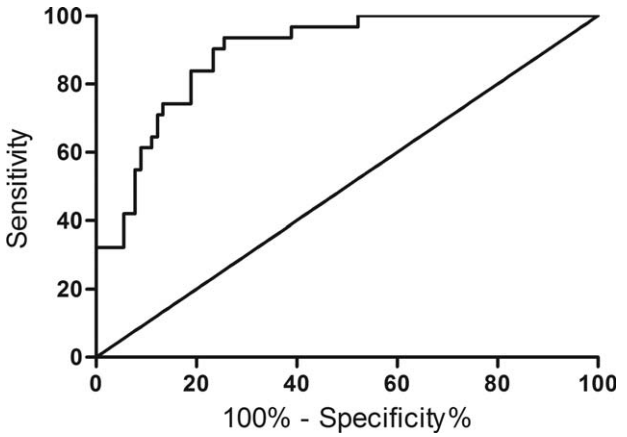

Figure 3. Receiver operating characteristic curve of serum human epididymis protein 4 as a marker for liver fibrosis.

serum HE4 and the amount of alcohol consumed (r = 0.031, $P = .890$) or length of alcohol use (r = 0.042, $P = .848$).

Results indicated that no significance in serum HE4 levels was found among hepatic patients(infected with HCV, NASH, or HBV) without fibrosis [44.12 (32.25, 64.11) pmol L^{-1} vs 50.42 (44.45, 55.22) pmol L^{-1} vs 65.65 (50.08, 100.10) pmol L^{-1}, $P = .093$] and no significant relations were found between serum HE4 and length of infection(r = –0.49, $P = .821$). In addition, no significance in serum HE4 levels was found among chronic liver injury patients, acute liver injury patients and healthy controls ($P = .323$), as shown in Table 2.

However, no significant differences in serum HE4 levels were found for age, gender, and HBV DNA copies($P > .05$).

4. Discussion

Developing noninvasive serum markers to predict liver fibrosis is urgently needed. Here we showed that serum HE4 levels were higher in liver fibrosis patients than that of hepatic patients without liver fibrosis and healthy volunteers, respectively. Luo et al reported that serum HE4 levels were higher in renal fibrosis patients.[8] In a systemic review, Chen also noted that serum HE4 levels were elevated in renal fibrosis.[13] In addition, Raghu reported that serum HE4 was higher in idiopathic pulmonary fibrosis.[14] These results are similar to the results presented here, in relation to liver fibrosis. Thus, serum HE4 might be a potential marker for liver fibrosis.

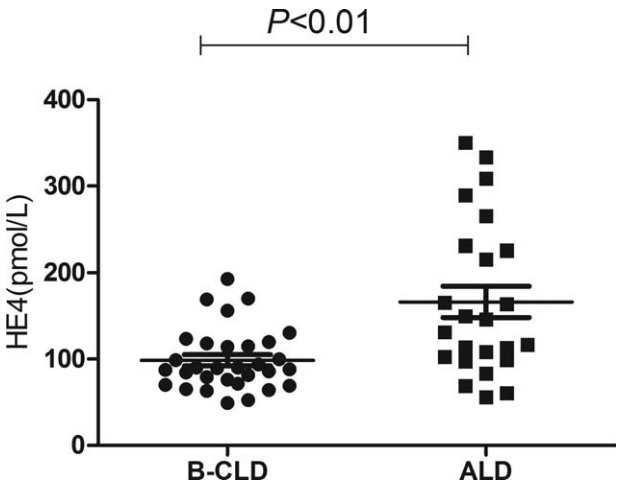

Figure 4. Serum human epididymis protein 4 levels between different etiologies of liver fibrosis.

Table 2

Relationships between serum human epididymis protein 4 and other Characteristics.

Characteristics	HE4 (pmol L^{-1})	P .323*
Chronic liver injury	51.00 (44.02,65.65)	.093
HCV	44.12 (32.25, 64.11)	
NASH	50.42 (44.45, 55.22)	
HBV	65.65 (50.08, 100.10)	
Acute liver injury	52.45 (46.02, 59.25)	
Healthy controls	46.2 (39.9, 58.9)	

* represents chronic liver injury vs acute liver injury vs healthy controls; HBV = Hepatitis virus B, HCV = Hepatitis virus C, HE4 = human epididymis protein 4, NASH = non-alcoholic steatohepatitis.

The C–P classification is commonly used to evaluate liver function in the context of chronic liver disease, mainly liver fibrosis.[15] Positive correlations were showed between serum HE4 levels and C–P scores. Serum HE4 levels were higher in ALD than that of chronic viral hepatitis B. Although no correlation was not found between the serum HE4 levels and different fibrosis scores, when serum HE4 levels in the various groups were compared individually, HE4 in F4 group were higher than that of F1, F2, or F3. It is interesting that higher HE4 levels were observed in the later stages of fibrosis. Statistical significance in this case might have been affected by the limited sample size. In future studies, we will enroll more fibrosis patients and investigate the relationship between serum HE4 and fibrosis score, as serum HE4 levels might serve as a marker for liver fibrosis.

ROC analysis indicated that the AUC of serum HE4 levels for liver fibrosis diagnosis was 0.921. A multicenter prospective cross-sectional diagnostic study showed that all the existing non-invasive serum markers for liver fibrosis diagnosis had a moderate accuracy in liver fibrosis diagnosis (AUC: 0.72–0.78).[16] In addition, serum hyaluronic acid and type IV collagen 7S are widely used for liver fibrosis in clinic practice; the AUC of serum hyaluronic acid and type IV collagen 7S for liver fibrosis was 0.752 and 0.798 respectively.[17] These results indicated that serum HE4 levels might have better diagnostic power than the existing serum markers.

Our study had some limitations: the sample size was a little small, which might affect the reliability of the conclusions. In future studies, we will enroll more patients in a multi-center study to provide enough data to confirm the findings of the present study.

In conclusion, elevated serum HE4 levels in liver fibrosis correlated positively with the C–Pclass and serum HE4 might be potential biomarker for liver fibrosis.

Author contributions

Conceptualization: Yulei Hou.
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Methodology: Fengzeng Li, Juanjuan Chen.
Project administration: Hui Chen
Supervision: Hui Chen
Validation: Hui Chen.
Writing – original draft: Yulei Hou.
Writing – review & editing: Hui Chen.

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