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Scandinavian Journal of Clinical and Laboratory Investigation

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/iclb20

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To cite this article: Nashwa Farouk Mohamed, Ola Galal Ali Behairy, Manal Sadek El defrawy, Samar Ahmed Mohamed Elsheraki & Rana Atef Khashaba (05 Dec 2024): Evaluation of serum human epididymis protein 4 in children with chronic liver diseases, Scandinavian Journal of Clinical and Laboratory Investigation, DOI: 10.1080/00365513.2024.2437612

To link to this article: https://doi.org/10.1080/00365513.2024.2437612

SCANDINAVIAN JOURNAL OF CLINICAL AND LABORATORY INVESTIGATION
https://doi.org/10.1080/00365513.2024.2437612

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RESEARCH ARTICLE

Evaluation of serum human epididymis protein 4 in children with chronic liver diseases

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ABSTRACT

The aim of this study was to evaluate the role of serum human epididymis protein 4 (HE4) as a non-invasive biomarker for the diagnosis of liver fibrosis in children with chronic liver diseases (CLD). This case-control study was conducted at Benha University Hospital, Egypt, involving 60 children with CLD and 60 healthy children as a control group. HE4 levels were measured by ELISA and compared with liver biopsy results. The CLD group had significant higher HE4 (median: 110.7, IQR: 96.7–120.4 pmol/L) compared to control group (median 42.07, IQR: 41.67–43.05 pmol/L), $p < .001$. HE4 levels increased significantly with the degree of fibrosis and histological activity index. At a cutoff point >48.3 pmol/L, HE4 diagnosed cases with mild fibrosis with a sensitivity of 95% and specificity of 91.3%. At a cutoff point >144.3 pmol/L, HE4 diagnosed cases with severe fibrosis with a sensitivity of 98% and specificity of 93.1%. Serum HE4 is a potential non-invasive marker for detecting liver fibrosis and its severity in children with CLD.

ARTICLE HISTORY
Received 14 October 2024
Revised 13 November 2024
Accepted 30 November 2024

KEYWORDS
Chronic liver disease; HE4; liver fibrosis; non-invasive biomarker; pediatric liver disease

Introduction

Chronic liver diseases in pediatric patients represent a significant medical challenge, characterized by frequent hospital admissions, enduring morbidity, and the risk of a fatal prognosis in the absence of definitive or palliative interventions [1]. The prevalence of hepatic disorders in the infant population stands at approximately 1 per 2500 live births. Notably, in 2021, pediatric cases accounted for 13% of the total liver transplantation procedures conducted in the United States [2].

Pediatric CLDs exhibit considerable heterogeneity, encompassing an extensive spectrum of pathologies that include congenital anomalies, metabolic dysfunctions, autoimmune conditions, and viral infections. Persistent hepatic insults in children often have the propensity to advance to varying stages of fibrosis and cirrhosis [3].

Hepatic fibrosis is characterized by the substitution of normal liver parenchyma with excessive extracellular matrix (ECM), occurring as a reparative mechanism in response to sustained hepatic or biliary tract damage. This mechanism involves the progressive deposition of fibrillar collagens, notably types I and III, alongside fibronectin, and anomalous elements of the extracellular matrix, such as proteoglycans enriched with chondroitin sulfate. These constituents collectively lead to the disorganization of the hepatic microarchitecture. Consequently, this disarray critically hinders the bidirectional plasma exchange between the sinusoidal spaces and hepatocytes, thereby exacerbating hepatic dysfunction [4].

The pathophysiological mechanisms underlying fibrosis have been extensively studied, particularly with respect to novel biomarkers and antifibrotic therapies, predominantly within the adult demographic. However, there is a paucity of knowledge regarding how these mechanisms manifest differently in pediatric patients, who possess a unique hepatic and immunological environment. Furthermore, certain pediatric-specific pathologies exhibit distinct fibrosis progression rates. For example, while fibrosis typically develops over several months in all patient groups, neonates experience an accelerated timeline [5].

The etiology of hepatic fibrosis frequently necessitates ongoing monitoring to establish appropriate therapeutic objectives. Despite its inherent drawbacks, such as its invasive nature, the possibility of adverse outcomes, and the hazard of inadequate sample retrieval, liver biopsy remains esteemed as the definitive gold-standard technique for this diagnostic objective.

The utilization of non-invasive serum biomarkers for the detection of hepatic fibrosis is on the rise. However, current biomarkers exhibit insufficient specificity and sensitivity, particularly in the early stages of fibrosis. Consequently, there is an urgent need for the identification of more reliable markers for early hepatic fibrosis [6].

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Human epididymis protein 4 (HE4), also referred to as the four-disulfide core domain protein 2 (WFDC2), is a secreted protein widely employed as a biomarker for ovarian carcinoma [7]. Recent research has revealed that HE4 is significantly involved in the mechanisms of fibrosis [8]. And Zhang et al. [9], demonstrated that hypoxia facilitates the accumulation of extracellular matrix and the progression of renal fibrosis through the upregulation of HE4 expression in tubular epithelial cells.

The aim of this study is to ascertain the utility of serum HE4 as a non-invasive biomarker in the diagnosis of liver fibrosis among pediatric patients afflicted with CLD.

Materials and methods

Study design and participants

This case control study was carried out on 60 children with chronic liver disease, who attended Gastroenterology and hepatology unit, Pediatric department, Benha University Hospitals, and 60 healthy children with age and sex matched as a control group, during the period from the first of April 2022 to the end of March 2023. The inclusion parameters for the study encompassed patients between the ages of 1 and 18 years, presenting with CLD of diverse origins. These included autoimmune hepatic diseases, cholestatic liver conditions, chronic viral hepatitis B and/or C, and metabolic hepatic disorders. Exclusion criteria were acute liver disease, solitary or multiple hepatic focal lesions, and CLD patients with comorbid conditions affecting the renal, cardiovascular, or central nervous systems.

Regarding controls; they were healthy children aged (1-18 years) without current illness or history of any chronic morbidity.

The sample size was calculated using Epi Info V.7. A previous study in adults reported that the serum HE4 levels in the fibrosis group was [105.35 (82.64, 164.18) pmol L^{-1}] while in the control group was [46.2 (39.9, 58.9) pmol L^{-1}] [6]. This difference between the groups was taken for calculating the sample size. At 95% level of significance and power of 80%, the minimal required sample size calculated was 33 in each group.

Ethical consideration

Informed written consent was obtained from the parents of all participating children, ensuring their voluntary involvement in the study. The study protocol was ethically sanctioned by the Benha University Ethical Committee, reference number Ms.9.3.2022.

Methods

All participating children underwent comprehensive history taking, thorough clinical examination, and a battery of laboratory tests. CBC were conducted using the Sysmex KX-21N analyzer (Sysmex Corporation, New York, USA). Liver function tests, including transaminases (AST, ALT), gamma-glutamyl transpeptidase, alkaline phosphatase, total and direct bilirubin, and serum albumin levels, were performed with the Biosystem A1A-auto analyzer (Spain). For children suspected of autoimmune hepatitis, gammaglobulin levels, anti-smooth muscle antibody (ASMA), and anti-liver kidney microsomal (Anti LKM) antibody levels were evaluated. Hepatitis markers (HBsAg, HCV) were also assessed. Additionally, serum HE4 levels were measured using a human epididymis protein 4 enzyme- ELISA kit (Human WAP Four Disulfide Core Domain Protein 2 (WFDC2), Catalog No: DL-WFDC2-Hu).

A Menghini aspiration needle (Hepafix Luer Lock, Braun Melsungen AG, Melsungen, Germany) was used to conduct ultrasound-guided liver biopsies on all study participants. Each biopsy procured an adequate core, encompassing no fewer than 11 portal tracts. The retrieved samples were promptly preserved in formalin, then embedded within paraffin for subsequent examination. Sections measuring five micrometers thick were meticulously prepared, affixed to glass slides, and subjected to hematoxylin and eosin staining. This process was undertaken to evaluate the histological activity of hepatitis, employing the Metavir scoring system for systematic assessment [10]. Additionally, Masson's trichrome staining was employed to evaluate the stage of fibrosis. Iron deposition was identified using Perls' Prussian blue stain, and PAS staining was conducted to exclude alpha-1 antitrypsin (A1AT) deficiency.

For predicting the prognosis of end stage liver disease, many prognostic models were proposed as:

Conceived by Child and Turcotte in 1964, the Child-Pugh scoring system was designed to streamline the selection process for candidates undergoing elective portal decompression surgery. It divides patients into three categories: A (indicating excellent hepatic function), B (indicating moderately impaired hepatic function), and C (indicating advanced hepatic dysfunction). Initially, the system implemented five clinical and laboratory parameters: serum bilirubin, serum albumin, ascites, neurological impairment, and nutritional status [11].

The Pediatric End-Stage Liver Disease (PELD) score, specifically designed for children under 12 years of age, was utilized to evaluate the necessity for liver transplantation and its impact on growth. This score is valuable for predicting the expected mortality rate associated with hepatic impairment in this population [12].

$$\begin{aligned}\textbf{PELD} = {} & 4.80\left[\text{Ln serum bilirubin}\left(\text{mg}/\text{dL}\right)\right]+18.57\left[\text{Ln INR}\right] \\ & -6.87\left[\text{Ln albumin}\left(\text{g}/\text{dL}\right)\right]+4.36\left(<1\,\text{year old}\right) \\ & +6.67\left(\text{growth failure}\right)\end{aligned}$$

MELD score ***(Model for end stage liver disease score)*** has proven effective in assessing prognosis and prioritizing liver transplant candidates. The calculation follows this formula [13]:

$$\begin{aligned}\text{MELD} = {} & 3.78\left[\text{Ln serum bilirubin}\left(\text{mg}/\text{dL}\right)\right]+11.2\left[\text{Ln INR}\right] \\ & +9.57\left[\text{Ln serum creatinine}\left(\text{mg}/\text{dL}\right)\right]+6.43.\end{aligned}$$

Statistical analysis

The dataset was methodically organized and subsequently analyzed through statistical methodologies using SPSS software version 16 (SPSS Inc., Chicago, IL). Data were presented and suitable analysis was done according to the type of data obtained for each parameter. Normality of data; Shapiro test was done to test the normality of data distribution. Descriptive statistics: Mean, Standard deviation (± SD) for parametric numerical data, while Median and IQR for non-parametric numerical data. Frequency and percentage of non-numerical data. Analytical statistics: Student t-test was used to assess the statistical significance of the difference between the two study group means. For the comparison of more than two groups' means, one way analysis of variance (ANOVA) was used. Mann–Whitney Test (*U* test) was used to assess the statistical significance of the difference of a non-parametric variable between two study groups. The Kruskal–Wallis test (K) was used to assess the statistical significance of the difference between more than two study group non parametric variables. The chi-Square test was used to examine the relationship between two qualitative variables. Fisher's exact test: was used to examine the relationship between two qualitative variables when the expected count is less than 5 in more than 20% of cells. Spearman's rank correlation coefficient (rho) was employed to elucidate the relationships between non-parametric variables. The criterion for statistical significance was rigorously set at 0.05, with values of $p < .05$ being deemed statistically significant.

Results

The 60 included CLD group children were 31 females and 29 males, their mean age was 10.7 ± 2.9 years, the median disease duration was 5 years (IQR: 5–10), 40% of patients had positive consanguinity. There was no statistically significant difference between the CLD group and control group regarding to age, sex or consanguinity, Table 1.

The most common complain of CLD patients was abdominal pain (50%), followed by jaundice (30%). 31.7% of patients had pallor, 3.3% had ascites and 6.7% had lower limb edema. The most common diagnosis in the studied patients was glycogen storage disease (50%), followed by autoimmune hepatitis (16.7%) and Wilson's disease (15%). Regarding liver biopsy, Most patients (53.3%) had mononuclear inflammatory cells, 31.7% had lymphocytes, 11.7% had lymphocytes and plasm cells and 3.3% had eosinophils. Regarding the degree of fibrosis; Most cases (53.3%) had mild (F1) fibrosis. Regarding Histological Activity Index, most cases (70%) had an A1 degree.

CLD group had significant higher HE4 (median: 110.7, IQR: 96.7–120.4 pmol/L) compared to the control group (median 42.07, IQR: 41.67–43.05 pmol/L), $p < .001$, Figure 1.

HE4 increased significantly with an increase in the degree of fibrosis and Histological activity index. While there was no statistically significant in HE4 levels in patients as regards to cell types, Table 2.

There was no statistically significant difference in human epididymis protein 4 (HE4) as regards to clinical diagnosis, Table 3.

There a statistically significant positive correlation between HE4 and (total bilirubin, direct bilirubin, ALT, AST, PT, INR, GGT, alkaline phosphatase, liver span, Child-pugh score, PELD score and MELD score) and a statistically significant negative correlation between HE 4 and (platelets and albumin), Table 4.

Discussion

Recently, a substantial number of publications have emerged exploring the diagnostic utility of HE4 levels across various conditions, including renal fibrosis and chronic kidney disease, cystic fibrosis, lung cancer, and the assessment of pulmonary dysfunction severity. However, to the best of our knowledge, this is the first study to assess serum HE4 in children with CLD. However, it was evaluated previously in adults with CLD.

Our results are in concordance with the findings of Hou et al. [6], who explored the clinical ramifications of serum HE4 in the context of adult liver fibrosis. Their study delineated that the HE4 concentrations in patients with liver fibrosis were 105.35 (82.64, 164.18) pmol L^{-1}, in hepatic patients devoid of fibrosis were 51.00 (44.02, 65.65) pmol L^{-1}, and in healthy control subjects were 46.2 (39.9, 58.9) pmol L^{-1}. Subsequent analyses elucidated those patients with liver fibrosis presented with significantly elevated serum HE4 levels relative to both healthy

Table 1. Socio-demographic data of the studied group.

		CLD group		Control group			*p*
		N = 60	%	*N* = 60	%	Test	value
Age (years)	Mean ± SD	10.7 ± 2.9		9.8 ± 3.2		*t* = 1.9	.09
	Range	1–17		1–17			
Sex	Female	31	51.7%	34	56.7%	X^2 = 0.30	.68
	Male	29	48.3%	26	43.3%		
Consanguinity	Negative	36	60.0%	45	75.0%	X^2 = 2.7	.09
	Positive	24	40.0%	15	25.0%		
Disease duration (years)	Median	7					
	IQR	5–10					

t: Student t-test; X^2: Chi-square test.

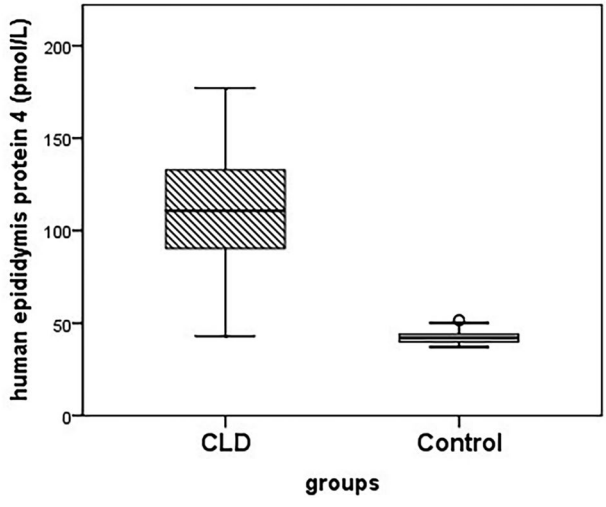

Figure 1. HE4 in The studied groups.

Table 2. Human epididymis protein 4 according to histopathological examination.

		Human epididymis protein 4 (pmol/L)			
		Median	IQR	Test	*p* value
Cells	Eosinophils	126.5	117–177.6	*K* = 1.2	.07
	Lymphocytes	115.7	90.4–124.8		
	Lymphocytes and plasma cells	137.8	116.8–170.5		
	Mononuclear inflammatory cells	101.2	94.8–113.7		
Degree of fibrosis	F1	89.8	83.5–99.2	*K* = 16.7	<.001*
	F2	110.7	95.1–120.4		
	F3	137.7	134.7–153.6		
	F4	169.6	141.2–176.5		
HAI	A1	99.3	91.8–124.9	*K* = 18.5	<.001*
	A2	135.7	132.2–145.5		
	A3	169.6	144.5–176.7		

HAI: Histological Activity Index; K: Krauscal Wallis test.
*significant.

Table 3. Human epididymis protein 4 according to clinical diagnosis.

		Human epididymis protein 4 (pmol/L)			
		Median	IQR	Test	*p* value
Diagnosis	Autoimmune hepatitis	120.5	99.3–144.9	*K* = 2.3	.09
	Biliary atresia	176.5	156.8–177.6		
	Chronic hepatitis C	122.9	81.4–150.4		
	Congenital hepatic fibrosis	149.7	98.7–164.7		
	Glycogen storage disease	120.6	91.9–130.7		
	Wilson's disease	137.8	133.4–168.9		

K: Krauscal Wallis test.
*significant.

Table 4. Correlation between HEP 4 and anthropometric measures and laboratory investigations in CLD group.

	Human epididymis protein 4 (pmol/L)	
	R	*p* value
Age (years)	0.134	.215
Disease duration (years)	0.112	.394
Weight centile	−0.074	.575
Height centile	−0.095	.471
BMI centile	−0.084	.658
Hemoglobin (g/l)	−0.095	.213
TLC ($\times 10^9$/L)	−0.047	.610
Platelets ($\times 10^9$/L)	−0.317	<.001*
Total bilirubin (μmol/L)	0.565	<.001*
Direct bilirubin (μmol/L)	0.571	<.001*
ALT (IU/L)	0.377	<.001*
AST (IU/L)	0.383	<.001*
PT (sec.)	0.304	.001*
PTT (sec	0.182	.113
INR	0.585	<.001*
GGT (U/L)	0.303	.019*
Alkaline phosphatase (U/L)	0.551	<.001*
Total IgG	0.096	.470
Albumin (g/L)	−0.527	<.001*
Liver span (cm)	0.298	.029*
Spleen size (cm)	0.022	.971
Child Pugh score	0.647	<.001*
PELD score	0.702	<.001*
MELD score	0.730	<.001*

r: Correlation coefficient; TLC: total leucocytes count; ALT: alanine aminotransferase, AST: aspartate aminotransferase; PT: prothrombin time; PTT: Partial thromboplastin time; INR: international normalized ratio; GGT: Gamma-Glutamyl Transferase; HAI: Histological Activity Index; PELD: Pediatric End-Stage Liver Disease; MELD: Model for End-stage Liver Disease.
*Significant.

controls (p = .00) and hepatic patients without fibrosis (p = .001), although the latter two groups did not exhibit significant differences (p > .05). Furthermore, a positive correlation was identified between serum HE4 concentrations and Child-Pugh scores. Notably, patients suffering from ALD demonstrated markedly higher serum HE4 levels compared to those with chronic viral hepatitis B. Despite the absence of a direct association between serum HE4 levels and various fibrosis scores, intergroup comparative analysis indicated that HE4 levels were substantially higher in the F4 fibrosis stage compared to the F1, F2, and F3 stages, thereby suggesting an intriguing pattern of elevated HE4 concentrations in advanced fibrosis stages. The researchers posited that further investigations, incorporating a larger cohort of fibrosis patients, are requisite to comprehensively elucidate the potential of serum HE4 as a biomarker for liver fibrosis, as the narrow sample size may have constrained the statistical significance of their findings. Additionally, a significant differential was observed in serum HE4 levels between patients with ALD-related fibrosis and those with fibrosis attributable to chronic HBV infection (p = .003). The ROC curve analysis for serum HE4 levels in fibrosis detection yielded a notable AUC of 0.921 (95% CI: 0.880–0.962), with an optimal threshold value of 69 pmol L^{-1}, indicating a sensitivity of 87.5% and a specificity of 81.1%.

Conversely, in the study by Zhang et al. [14], undertook a study investigating HE4 concentrations within the framework of liver fibrosis and cirrhosis. Their investigation disclosed that the median serum HE4 concentrations in patients with chronic liver disease (median: 56.2, IQR: 39.2–76.0 pmol/L) were statistically analogous to those in the matched control cohort (median: 55.0, IQR: 39.5–86.8 pmol/L; p = .562), with no significant disparity observed (p = .562). Additionally, the data indicated no substantial correlation between serum HE4 levels and the extent of histological liver fibrosis (r = 0.045, p = .788). Moreover, there was no statistically significant association between the Child-Pugh score and HE4 concentrations in the patient population (r = 0.007, p = .964).

Table 5. Summary of all studies published in this direction.

Author	Year	Study design	No. of patients	findings	Conclusion
Hou et al. [6]	2020	Case control	65 liver fibrosis patients, 68 hepatic patients without fibrosis, and 50 controls	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 control Serum HE4 levels in liver fibrosis patients with C–P class C were higher than that of C–Pclass A	elevated serum HE4 levels in liver fibrosis correlated positively with the C–Pclass and serum HE4 might be potential biomarker for liver fibrosis
Zhang et al. [14]	2018	Case control	A total of 366 consecutive patients with chronic liver disease	No statistically significant differences were observed in the median of HE4 levels between patients with CLD (median: 56.2, interquartile range (IQR): 39.2–76.0 pmol/L) and matched controls (median: 55.0, IQR: 39.5–86.8 pmol/L; p = .562), nor was there any evidence of a relevant trend towards higher levels of HE4 among the advanced fibrosis groups	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
Yu et al. [21]	2024	Case control	A total of 109 patients were enrolled in this study	The serum HE4 levels showed significant differences among patients with F0, F1, F2, F3, and F4 [50.71 (40.48–59.03) vs 60.69 (51.40–68.80) vs 73.20 (69.05–79.10) vs 80.72 (75.08–90.98) vs 98 (74.02–155.00) pmol/L, p = .004]. The subsequent analysis revealed significantly elevated serum HE4 levels in patients with F4 compared to those with F0	Serum HE4 levels were found to be elevated in AIH-LC patients and exhibited a strong correlation with the severity of hepatic fibrosis, thus supporting their potential clinical value as a novel biomarker of disease severity and hepatic fibrosis in AIH.

Recent investigations have elucidated that HE4, alternatively identified as Whey Acidic Protein (WAP) 4-disulfide core domain 2, is uniquely upregulated in activated myofibroblasts and subsequently released into the bloodstream. This biomarker, HE4, serves a pivotal role in the activation of fibroblasts and the facilitation of extracellular matrix accumulation. Quantitative assessments of HE4 levels in the serum of individuals afflicted with renal pathologies demonstrate a significant association with the extent of renal fibrosis. Moreover, experimental administration of anti-HE4 antibodies in murine models has been evidenced to ameliorate renal fibrosis, ostensibly by mitigating the multifaceted protease inhibitor activity inherent to HE4 [15].

HE4 has emerged as a preeminent and innovative serum biomarker with substantial potential for the diagnosis, prognostication, and surveillance of various pathological conditions, with its diagnostic efficacy receiving endorsement and validation from the Food and Drug Administration. Within the vicinity of HE4, two extensively studied co-expressed genes, namely the secretory leukocyte protease inhibitor (SLPI) and P13, have been identified. These genes exhibit a multifaceted range of biological activities including regulation of angiogenesis, modulation of cellular proliferation and migration, and involvement in immune responses, antimicrobial actions, and anti-HIV properties [16].

Luo et al. [17] previously documented elevated serum HE4 concentrations in patients suffering from renal fibrosis. Similarly, a comprehensive review by Chen et al. [18] corroborated the elevated serum HE4 levels in the context of renal fibrosis. Furthermore, Raghu et al. [19] observed increased serum HE4 in cases of idiopathic pulmonary fibrosis. Additionally, Piek et al. [20] posited that augmented HE4 levels could potentially promote cardiac fibrosis, thereby exacerbating fibrosis-induced end-organ damage in the context of heart failure. Taken together, these studies highlight the pivotal role of HE4 in the etiopathogenesis of fibrotic processes (Table 5).

The findings reported herein are congruent with those observed in the context of hepatic fibrosis, suggesting that serum HE4 may serve as a viable biomarker for liver fibrosis. Elevated levels of serum HE4 in liver fibrosis exhibit a positive correlation with the extent of fibrosis and the severity of the disease, independent of the etiology of CLD. Consequently, serum HE4 levels may possess robust diagnostic potential for assessing fibrosis in pediatric patients with CLD.

Finally, this study faced certain limitations, primarily the relatively small sample size, which may impact the robustness of the derived conclusions. Future research should involve larger, multi-center studies to generate sufficient data and substantiate the findings of this investigation.

In conclusion, serum HE4 has the potential to serve as a credible biomarker for liver fibrosis. Elevated serum HE4 concentrations in cases of liver fibrosis demonstrated a positive correlation with the extent of fibrosis and disease severity, independent of the underlying etiology of CLD. Thus, serum HE4 levels may possess significant diagnostic utility for detecting fibrosis in pediatric patients with CLD.

Acknowledgements

For their unending dedication, technical assistance, and tolerance, the entire team at Benha University's Pediatric Gastroenterology and Hepatology section deserves our deepest admiration and appreciation. They were essential to the success of this investigation. Additionally, we would like to express our gratitude to the CLD individuals and the healthy volunteers who participated by providing the necessary information and samples with such good humor and dedication.

Ethical approval

The study was approved by the Institutional Ethical Committee, Faculty of Medicine, Benha University. Informed consents were obtained from the parents or the guardians.

Consent for publication

All authors give their consent for publication; they all have agreed to publish this work.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

None to be declared.

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Data availability statement

The data is available upon reasonable request from the authors.

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