

## Diagnostic Value of Procollagen III N-terminal Peptide in Children with Chronic Liver Diseases

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### ABSTRACT

**BACKGROUND:** Liver fibrosis develops as a result of a variety of chronic liver diseases. The degree of liver fibrosis must be determined before the severity of the disease can be assessed. The gold standard approach is invasive liver biopsy which is linked with pain and complications. Direct measures of extracellular matrix turnover and indirect markers as a reflection of liver dysfunction are two types of biomarkers used to identify liver fibrosis.

**AIM:** This study aims to evaluate the diagnostic value of procollagen III N-terminal peptide (P-III-N-P) levels in children with chronic liver diseases.

**SUBJECTS AND METHODS:** Sixty children with chronic liver diseases and 30 healthy children as a control group were included

in this study. Medical history, clinical, radiological, laboratory investigations, plasma procollagen III N-terminal peptide levels were determined using ELISA kits were used to assess both groups, histopathological liver biopsy for case group only.

**RESULTS:** The hepatic group had a statistically significant higher level of procollagen III N-terminal peptide than the control group (median; 2029.5 ng/ml, 620 ng/ml respectively,  $p < 0.001$ ). In all studied cases, the mean  $\pm$  SD of APRI, FIB-4 scores, and procollagen III N-terminal peptide levels exhibited a significant tendency of increasing with the progressive fibrosis stage as determined by the histological Ishak scoring system. The level of procollagen III N-terminal peptide was positively correlated with serum biomarkers and the degree of fibrosis in all patients.

**CONCLUSION:** Serum procollagen III N-terminal peptide level is a useful non-invasive marker for detection of the stage of liver fibrosis in children with chronic liver diseases.

**Key words:** Procollagen III N-terminal peptide; Children; Chronic liver diseases; Liver fibrosis

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### INTRODUCTION

Chronic liver disease (CLD) is defined as six months or longer period of progressive impairment of liver functions. CLD is a continual process of liver parenchyma inflammation, destruction, and regeneration that leads to fibrosis and cirrhosis. Chronic hepatitis and liver cirrhosis are among the liver pathologies that make up CLD<sup>[1]</sup>.

Because it gives predictive information and, on many occasions, contributes to therapeutic decisions, grading liver fibrosis is considered to be an important aspect of the management of patients with CLD<sup>[2]</sup>. Although liver biopsy is the gold standard for determining the type and grade of liver fibrosis, it is still with many limitations as it is a very painful procedure. Furthermore, poor

specimen quality as well as tissue size make biopsy non-reproducible and depend on the pathologist's experience, resulting in inter-observer differences. Pain (84%), hypertension (0.5%), and biliary system injury (0.5%) are all complications due to liver biopsy, with a 0.01% percentage fatality risk<sup>[3]</sup>. Because of the limitations of liver biopsy, the development of a noninvasive diagnostic procedure for hepatic fibrosis has become urgent<sup>[4]</sup>.

Extracellular matrix (ECM) with glycoproteins, proteoglycans, and glycosaminoglycans are found in the space of Disse in the normal liver in touch directly with the low-density basal lamina. Necrotic or apoptotic cells are replaced by regenerated parenchymal cells after an acute hepatic injury. If the hepatic damage is persistent, recovery will fail and hepatocytes will be replaced with excessive ECM and fibrillar collagen<sup>[5]</sup>. Liver fibrosis is linked to numerous changes in ECM amount and composition. Collagens (I, III, and IV), fibronectin, elastin, laminin, hyaluronic acid (HA), and proteoglycans make up three to ten times more ECM in the advanced fibrotic liver than in normal liver<sup>[6]</sup>.

Procollagen undergoes enzymatic cleavage at the carboxy and amino final ends by procollagen C-peptidase and procollagen N-peptidase during production, producing peptides into the serum that can be used to evaluate matrix accumulation<sup>[7]</sup>. Type III collagen is fibril-forming collagen that is present in connective tissue. During hepatic fibrosis caused by chronic liver injury, levels of procollagen III amino peptide (P-III-N-P) in the basal membrane are elevated<sup>[8]</sup>. The purpose of the study was to determine if procollagen III N-terminal peptide (P-III-N-P) levels could be used to evaluate the hepatic fibrosis in chronic liver diseases in children.

## METHODS

### Study design and population

A total of 90 children were studied in this case-control study, and they were divided into two groups: based on clinical, laboratory, and histopathological tests, sixty children were diagnosed with (a long-term irreversible alteration in the liver structure that can cause serious problems such as cirrhosis and early mortality)<sup>[9]</sup> between December 2020 and December 2021, patients were selected from Benha University Hospitals' pediatric hepatology outpatient clinic, along with 30 age/gender-matched healthy children who participated as the control group. Children who went to an outpatient clinic for a routine checkup for athletic training or a school routine evaluation participated as control subjects. Patients with chronic viral hepatitis B or/and C, cholestatic liver disease, autoimmune hepatitis, or metabolic hepatic disorders were included in the research. Any patient with CLD who had a co-morbidity (renal, CVS, or CNS disorder) was eliminated. The research was approved by the Ethical Committee of Benha University's Faculty of Medicine, which followed "The World Medical Association's Code of Ethics" (Declaration of Helsinki). Parents/guardians gave their consent after being properly informed about all study protocols before enrolment.

All of the children who were enlisted underwent a full history taken and clinical examination, with a focus on clinical symptoms (jaundice, abdominal cramping and distension, melena, and hemorrhagic symptoms such as hematemesis, melena, or bleeding gums), abdominal ultrasonography, and laboratory tests, which included:

1. Complete blood picture by Sysmex-XP300, international normalized ratio (INR), prothrombin time (PT), activated partial thromboplastin time (aPTT) were done by TEClot PT-S on Coatron A4 instrument (Germany), and liver function tests; aspartate

aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), total and direct bilirubin and serum albumin were done by Biosystem A1A-auto analyser-Spain.

2. Serum immunoglobulin G (IgG): By radial immunodiffusion using (IgG-NLRID, RN004.3, Binding Site, Birmingham, United Kingdom).

3. Serum autoantibodies which include: anti-nuclear antibodies (ANA), anti-smooth muscle antibodies (ASMA) titer, liver-kidney microsome antibodies (anti-LKM-1), Anti liver cytosol type 1 (LC1), and anti-mitochondrial antibodies (AMA) titer were performed by indirect immunofluorescence technique using NOVA Lite Rat Liver, Kidney, Stomach (INOVA Diagnostic Inc, Germany).

4. Serological tests for viral hepatitis B, and C: Hepatitis B surface antigen (HBs Ag), and anti-HCV antibody (HCV-Ab) by enzyme-linked immunosorbent assay (ELISA) third generation, using the kit from Abbott laboratories (Wiesbaden, Delknheim, Germany) and confirmation was done by using PCR (Biokit, Spain).

5. Work-up for Wilson's disease (serum ceruloplasmin, 24-h urinary copper before and after penicillamine) and slit-lamp examination for Keyser-Fleischer ring.

6. Work-up for Tyrosenemia by (succinylacetone in blood and urine and alpha-fetoprotein).

7. Work-up for Neiman pick by (bone marrow aspiration and fundus examination).

8. Serum procollagen III N-terminal peptide (P-III-N-P) levels were measured using human enzyme-linked immunosorbent assay (ELISA) (sandwich technique) kits (Bioassay technology, Zhejiang, China) (Cat.No.:E-EL-H0183).

9. Additional surrogate blood indices of liver fibrosis: were calculated according to the published analytic recommendations<sup>[10-11]</sup> as follows: (1) Aspartate aminotransferase to platelet ratio index (APRI), the APRI was calculated as follows:  $APRI = ((AST/upper\ limit\ of\ a\ normal\ range\ of\ AST) \times 100) / platelet\ count\ (10^9/L)$ ; (2) Fibrosis-4 (FIB-4) =  $[age\ (yrs) \times AST\ (IU/L)] / [platelets\ count\ (10^9/L) \times \sqrt{ALT\ (IU/L)}]$ .

Only the CLD group got an ultrasound-guided liver biopsy using a Menghini suction needle to obtain a sufficient core that has at least 11 portal tracts (Hepafix Luer Lock Braun Melsungen AG, Melsungen, Germany). Formalin-fixed, paraffin-embedded biopsy tissue was split and stained with hematoxylin and eosin to evaluate the histological activity of liver disease Using the Ishak scoring system: F0 (no fibrosis), F(1-2/6) (Fibrous expansion of some or most portal areas, with or without short fibrous septa), F(3-4/6) (Fibrous expansion of most portal areas with occasional or marked bridging (portal-portal and portal-central), F(5-6/6) (Marked bridging (portal-portal and/or portal-central) with occasional nodules (incomplete cirrhosis) or cirrhosis)<sup>[12]</sup>. Mason-Trichrome stain was used to determine the level of fibrosis, Perls' Prussian blue stain was used to detect iron deposition, and the periodic acid chief stain was used to rule out alpha 1 anti-trypsin deficiency. The slides were examined blindly by two pathologists.

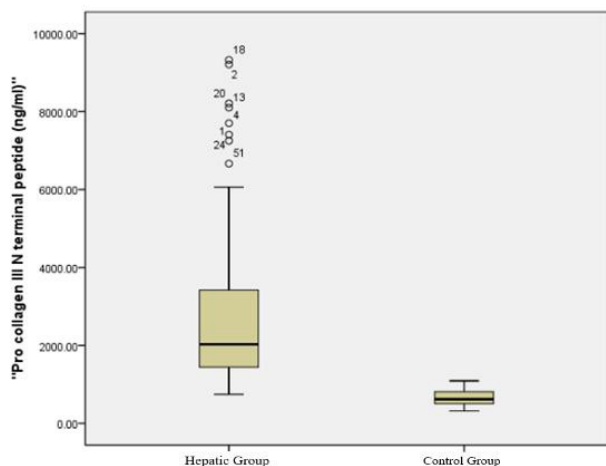
### Statistical Methods

SPSS software, version 16, was used to tabulate and analyze the collected information (Spss Inc., Chicago, IL, U.S.A.). Number (percent) was utilized to characterize qualitative data, and the chi-square (2) test was employed to compare them. The mean and standard deviation were used to illustrate quantitative information (SD). To compare two or more groups, the Student (t) and ANOVA (F) tests were used, respectively. To evaluate correlations between variables,

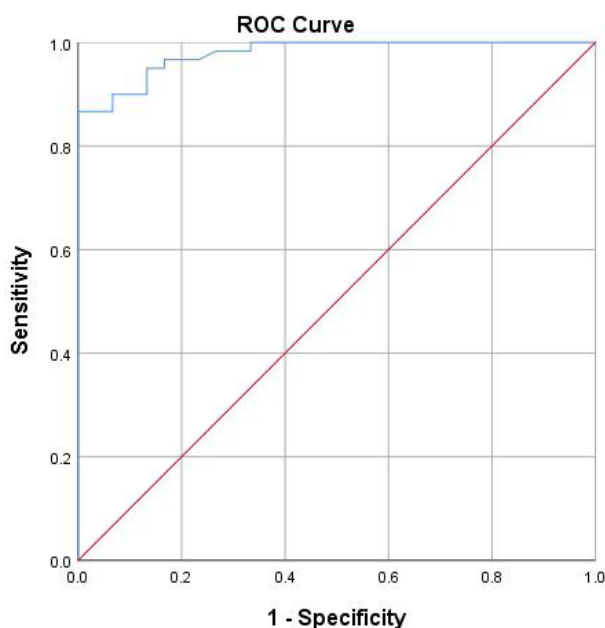
Pearson’s correlation coefficient (r) was employed. In the diagnosis of early and extensive fibrosis, ROC curve analysis was performed to determine cut-off values of serum procollagen III N-terminal peptide (P-III-N-P) with the highest sensitivity and specificity. To evaluate fibrosis scores, researchers used multivariate analysis. The importance of the outcomes was recognized at a P value of 0.05 with a 95% confidence interval (P0.05 was considered important).

**Table 1** Distribution of histopathological fibrosis stages according to different etiologies of chronic liver diseases.

Diagnosis			Fibrosis stage		
			Mild F1 (1-2/6) (No.52)	Moderate F2(3-4/6) (No.6)	Severe F3 (5-6/6) (No.2)
Diagnosis	Cholestatic liver diseases (biliary atresia)	No. (%)	1 (1.9%)	0 (0%)	0 (0%)
	Congenital hepatic fibrosis	No. (%)	0 (0%)	0 (0%)	1 (1.9%)
	Autoimmune hepatitis	No. (%)	11 (21.2%)	1 (16.7%)	2 (100.0%)
	Glycogen storage disease	No. (%)	24 (46.2%)	2 (33.3%)	0 (0%)
	Wilson disease	No. (%)	5 (9.6%)	1 (16.7%)	0 (0%)
	Hepatitis C	No. (%)	8 (15.4%)	2 (33.3%)	0 (0%)
	Hepatitis B	No. (%)	2 (3.8%)	0 (0%)	0 (0%)



**Figure 1** Procollagen III N-terminal peptide among studied groups.



**Figure 2** Receiver operating characteristic (ROC) curve for the diagnostic performance for prediction of liver fibrosis for Procollagen III N-terminal peptide.

## RESULTS

There were 60 CLD children in this research, 28 boys and 32 girls, with an average mean age of  $10.13 \pm 3.33$  years and a range of 1 to 17 years. The 30 healthy children, who ranged in age from 1 to 17, were 14 boys and 16 girls with a mean age of  $9.46 \pm 2.87$  years. Both groups were matched in regards to sex and age ( $p = 1, 0.353$ , correspondingly), with no significant difference.

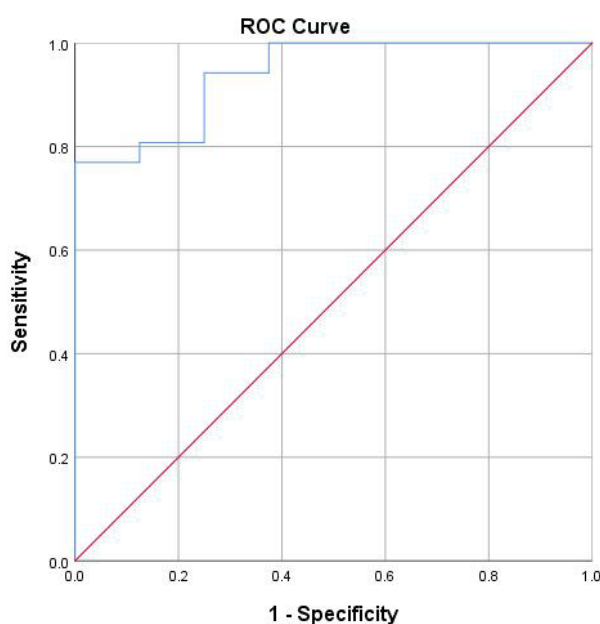
### Etiology and clinical presentation of CLD in the patient group

Comprehensive investigations were used to evaluate the CLD patients, and the basic causative agents were proven by liver biopsies, which also verified the actual fibrosis degree in each patient. (Table 1) Hereditary abnormalities [glycogen storage disorder 26 cases (43.3%), Wilson disorder 6 cases (10%), immune-mediated hepatitis type I 14 cases (23.3%), congenital liver fibrosis 1 case (1.6%), infective liver disease (HBV 2 cases (3.3%) and HCV 10 cases (16.7%), and cholestatic liver problems (biliary atresia 1 case (1.6%). Jaundice (16.7 percent), abdominal distention (38.3%), clay color feces (1.7 percent), abdominal cramps (16.7 percent), faltering growth (3.3 percent), Abdominal ultrasound imaging detected hepatomegaly in 85 percent of the cases and splenomegaly in 28.3 percent of patients having CLD. No cases had portal hypertension or ascites.

### The study participants’ laboratory findings and histopathological evaluation

The study’s baseline laboratory data found a significant difference in liver function tests (ALT, AST, PT, and bilirubin) among CLD patients compared to healthy controls, with CLD patients having greater levels (Table 2).

According to the Ishak scoring method<sup>[12]</sup>, disease activity was minor (1-3/18) in 30% of cases, mild (4-8/18) in 63.3 percent, moderate (9-12/18) in 1.7 percent, and extreme (13-18/18) in 5%. Meanwhile, 86.7% percent had mild fibrosis (F1-2/6), 10% had moderate fibrosis (F3-4/6), and 3.3 percent had extreme fibrosis (F5-6/6), in relation to the grade of fibrosis.



**Figure 3** Receiver operating characteristic (ROC) curve for the diagnostic performance of prediction of moderate/severe cases in CLD group for Procollagen III N-terminal peptide.

**Table 2** laboratory data of the studied groups.

Variables		Hepatic group (No= 60)	Control group (No= 30)	P-value
Total bilirubin (mg/dL)	Mean ± SD (Range)	1.41 ± 0.670 (0.60 - 5.10)	0.744 ± 0.187** (0.18 - 0.98)	0
Direct bilirubin (mg/dL)	Mean ± SD (Range)	1.25 ± 0.890 (0.30 - 5.00)	0.222 ± 0.092** (0.11 - 0.50)	0
ALT (IU/L)	Mean ± SD IQR Median	59.66 ± 75.36 (32 - 544) (41.50)	28.23 ± 5.34* (15 - 38) (29.00)	0
AST (IU/L)	Mean ± SD IQR Median	75.06 ± 94.23 (41- 534) (50.00)	28.46 ± 7.09* (12 - 40) (28.50)	0
GGT (IU/L)	Mean ± SD (Range)	40.77 ± 26.65 (2.6 - 167)	23.56 ± 5.37** (13 - 31)	0.001
Albumin (g/dL)	Mean ± SD (Range)	4.06 ± 0.451 (3 - 4.8)	4.11 ± 0.437** (3.5 - 4.9)	0.58
PT (seconds)	Mean ± SD (Range)	12.97 ± 0.740 (10 - 16.2)	12.49 ± 0.724** (11 - 14.5)	0.004
INR	Mean ± SD (Range)	1.03 ± 0.046 (1 - 1.13)	0.989 ± 0.070** (0.8 - 1.13)	0.001
Procollagen III N-terminal peptide (ng/ml)	Mean ± SD (Range) (Median)	2936.73 ± 2313.15 (748 - 9210) 2029.5	665.50 ± 208.87* (318 - 1092) 620	0.001

\*: U= Man-Whitney test, \*\*: t-test, ALT: alanine aminotransferase, AST: aspartate aminotransferase, PT: prothrombin time.

**Table 3** Comparison between the histopathological degree of fibrosis and non-invasive fibrosis scores (APRI and FIB-4) and serum Procollagen III N-terminal peptide levels in chronic liver disease patients.

Variable		APRI Score	Fib-4	Procollagen III N terminal peptide (ng/ml)	
Degree of fibrosis	Mild [n=(51)]	F 1-2/6	0.04 ± .001	0.411 ± 0.370	1799.32 ± 977.33
	Moderate [n=(6)]	F 3-4/6	0.284 ± .602	0.476 ± 0.311	3182.50 ± 1641.36
	Severe [n=(3)]	F 5-6/6	0.767 ± 0.694	1.04 ± 0.756	8230.50 ± 1385.22
F-test		15.809	2.735	5.31	
P-value		0	0.04	0	

**Table 4** Comparison between levels of Procollagen III N-terminal peptide as regards the diagnosis of the CLD group.

Diagnosis	Procollagen III N-terminal peptide (ng/ml)		F-test	P
	Mean ± SD	Range		
Diagnosis	Cholestatic liver diseases (biliary atresia)	2101	5.21	0.073
	Congenital hepatic fibrosis	1210		
	Autoimmune hepatitis	2807.14 ± 2387.29		
	Glycogen storage disease	1744.26 ± 985.73		
	Wilson disease	2391.50 ± 892.83		
	Hepatitis C	2299.10 ± 1958.18		
	Hepatitis B	1909.50 ± 1436.13		

**Hepatic fibrosis indicators that do not require invasive testing**

The use of non-invasive surrogate blood indices of hepatic fibrosis showed that the mean SD of APRI and FIB-4 scores (1.04 ± 0.85 and 0.439 ± 0.38 respectively) increased with increasing the severity of fibrosis levels evident with the histological Ishak scoring system (P 0.001) (Table 3).

**Evaluation of serum procollagen III N-terminal peptide levels**

CLD patients had significant greater serum procollagen III N-terminal peptide levels than control subjects (P 0.001) (Table 2, Figure 1).

The level of serum procollagen III N-terminal peptide rose significantly with increase the severity of fibrosis in CLD patients (Table 3).

Serum procollagen III N-terminal peptide levels did not differ significantly according to CLD etiologies in studied patients (p > 0.05) (Table 4).

Serum procollagen III N-terminal peptide levels was found to be positively correlated with AST (r= 0.198, p < 0.001), ALT (r = 0.197, p < 0.001), GGT (r = 0.257, p < 0.001), PT (r = 0.185, p < 0.001), total bilirubin (r = 0.378, p < 0.001), direct bilirubin (r = 0.363, p < 0.001), liver span (r = 0.225, p < 0.041), spleen size (r = 0.409, p < 0.001), APRI (r = 0.391, p < 0.002) and FIB-4 (r = 0.376, p < 0.003).

**Diagnostic performance and predictive value of serum procollagen III N-terminal peptide levels**

The diagnostic performances of procollagen III N-terminal peptide

**Table 5** Multi-linear regression analysis for predictors of fibrosis.

Variables	Coefficients			P-value
	Standardized Coefficients	95.0% Confidence Interval for B		
		Beta	Lower Bound	
Total Bilirubin	1.82	1.1	3.08	0.03
Procollagen III N terminal peptide (ng/ml)	3.42	1.9	5.54	0
APRI Score	1.71	1.04	2.86	0
AST	1.3	0.8	1.9	0.03
ALT	1.2	0.9	2.1	0.04
Fib-4	5.1	1.3	9.4	0

ALT = Alanine Aminotransferase, AST = Aspartate Aminotransferase, APRI score = AST to platelet ratio index, Fib-4 = fibrosis-4 index.

levels to detect the presence of fibrosis in children with CLD indicated that at cut off value ≥ 982 ng/ml had a sensitivity of 90% and specificity of 90%, with a fair area under the ROC curve (AUC) of 0.97.9 (Figure 2), and, at cut-off value of ≥ 2224 ng/mL cases of moderate/severe fibrosis could be differentiated from cases with mild fibrosis with AUC was 0.94 had sensitivity 80.8% and specificity was 87.5% (Figure 3).

AST, ALT, APRI, FIB-4, and procollagen III N-terminal peptide are all potential indicators of fibrosis in children with CLD, according to multiple linear regression analysis (Table 5).

**DISCUSSION**

Type III collagen is primarily found in the liver. Because the N-terminal pro-peptide of procollagen type III (P-III-N-P) is released from procollagen type III during the production of type III collagen, fibrogenesis occurs in the discharge of ECM fragments into the bloodstream. As a result, the level of (P-III-N-P) can be used as a direct measure of collagen formation and accumulation in the extracellular space<sup>[13]</sup>.

The mean procollagen III N terminal peptide level in the CLD category was statistically higher in the control group in the ongoing study. This was in line with a previous study that showed a statistically significant difference in procollagen III N-terminal peptide levels, and they concluded that serum procollagen III N-terminal peptide was the most effective for fibrosis degree assessment<sup>[14]</sup>. In chronic

hepatitis C and primary biliary cirrhosis, the procollagen III N-terminal peptide is a degradation product of collagen precursor and has been the most widely investigated non-invasive predictor of hepatic fibrosis<sup>[15,16]</sup>.

Type III collagen is fibril-forming collagen that is found in connective tissue. Procollagen III amino-peptide levels in the basal membrane are elevated during hepatic fibrosis resulting from chronic hepatic injury<sup>[17]</sup>.

The serum concentrations of procollagen III N-terminal peptide in the ongoing study were unaffected by the causes of CLD in the cases, but the severity of fibrosis elevated, procollagen III N-terminal peptide concentrations have risen dramatically; Furthermore, there was a strong positive link between procollagen III N-terminal peptide levels and (ALT, AST, PT, liver span, spleen size, APRI score, and FIB-4 score). Our results are in accordance with many other researches who found a link between serum procollagen III N-terminal peptide concentrations and histological liver fibrosis grade in adults<sup>[18-22]</sup>. Only two research on the pediatric population addressed this topic. The serum procollagen III N-terminal peptide concentration increased significantly with the degree of liver fibrosis, according to Wang *et al*<sup>[14]</sup> and Mosca *et al*<sup>[23]</sup>, and the connection between serum procollagen III N-terminal peptide concentration and hepatic fibrosis stage was powerful, positive, and straight. Multivariate ordinal regression findings showed that serum procollagen III N-terminal peptide was an independent predictor of liver fibrosis grade. These data suggest that higher serum procollagen III N-terminal peptide concentrations may have a greater grade of hepatic fibrosis, which is similar to previous adult studies.

The efficacy of procollagen III N-terminal peptide for CLD identification and fibrosis degree estimation was proven to be an excellent diagnostic marker for identifying mild fibrosis from normal subjects in the detection of extreme fibrosis in the current research. Mosca *et al*<sup>[23]</sup> evaluated the predictability of procollagen III N-terminal peptide in determining F 2 or F3 fibrosis in the same circumstance and AUCs of 92.1 percent and 99.3 percent were reported, respectively. Wang *et al*<sup>[14]</sup> confirmed the use of procollagen III N-terminal peptide, suggesting it to have a moderate diagnostic performance for fibrosis grade and the greatest AUC for diagnosing cirrhosis (89.1 percent ). While the F1 assessment took second place with an AUC of 84.3 percent, the F2 stage diagnosis had the lowest accuracy with an AUC of 78.9 percent. These findings showed that serum P-III-N-P was better at detecting liver fibrosis and cirrhosis than distinguishing between fibrosis stages, which is in consistent with our findings.

Also, Leroy *et al*<sup>[24]</sup> observed that the experimental procollagen III N-terminal peptide concentration was a real indicator of hepatic fibrosis grade in chronic hepatitis C patients, with an AUROC value of 0.88 for detection of stages F3 and F4.

Because of its small sample size, the recent study has limitations. Multiple points of strength, however, could be established. To the best of our knowledge, this is the first study to investigate the Procollagen III N terminal peptide levels and accuracy for identifying and classifying fibrosis of CLD in children with various etiologies. We also compared it to existing markers for fibrosis score and the gold standard for diagnosis, a liver biopsy. In addition, healthy children were included for comparison which enhanced validation of the results.

## CONCLUSION

The level of serum procollagen III N-terminal peptide increased significantly in correlation with the severity of liver fibrosis and

represents a promising, non-invasive biomarker for identifying hepatic fibrosis in children with chronic liver diseases.

## Declarations

Ethics approval and consent to participate: The current study was approved by the Medical Research Ethical Committee of the Faculty of Medicine, Benha University. All subjects were informed about the procedures and the aim of the study and informed written consent was obtained from the parents or caregivers of enrolled children. The committee's reference number is not available.

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**Authors' contributions:** 1.M S: Contributed to the design and implementation of the research, aided in choosing the patients and helped shape the research, supervised the findings of this work, discussed the results, read, and approved the final manuscript. 2.O B: Contributed to the design and implementation of the research, aided in choosing the patients and helped shape the research, supervised the findings of this work, discussed the results, read, and approved the final manuscript. 3.S A: Contributed to the design and implementation of the research, aided in choosing the patients, performed the laboratory work and helped shape the research, supervised the findings of this work, discussed the results, read, and approved the final manuscript. 4.S R: Contributed to the design and implementation of the research, aided in choosing the patients and helped shape the research, supervised the findings of this work, discussed the results, read, and approved the final manuscript.

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