

# The role of serum type IV collagen and serum procollagen type III (PIIINP) as biomarkers for the detection and staging of liver fibrosis in a sample of Iraqi patients with chronic liver disease

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## Abstract

**Background:** The standard diagnostic for grading and staging hepatic fibrosis and inflammation is still a liver biopsy. However, noninvasive techniques are now favored due to several limitations of the liver biopsy. In order to measure the degree of liver fibrosis in chronic liver disorders, some research suggested using hyaluronic acid and laminin as indicators.

**Aim of the study:** The aim of the current study was to evaluate the role of serum type IV collagen and serum procollagen type III (PIIINP) as biomarkers for the detection and staging of liver fibrosis in a sample of Iraqi patients with chronic liver disease.

**Patients and methods:** The current case control study included 100 patients with liver fibrosis and 50 healthy control individuals of comparable age and gender. The age range of patients was from 32 to 67 years. Patients were having chronic liver disease and they were grouped into 22, 36, 19 and 23 as having alcoholic liver disease, non-alcoholic liver disease, HBV and HCV. Demographic characteristics were obtained including age, gender and body mass index. Assessment of the following biochemical testes was performed to all participants by ELISA: ALT, AST, ALP, GGT, TSB, Albumin, type IV collagen and procollagen type III (PIIINP).

**Results:** There was significant difference in mean collagen IV among patients' and control groups ( $p < 0.001$ ) in such a way that highest level was reported in patients with grade 3-4 liver fibrosis and this level was significantly higher than that of patients with liver fibrosis grade 0-1 and control group ( $p < 0.05$ ) and the level of patients with liver fibrosis grade 0-1 was significantly higher than that of control group ( $p < 0.05$ ). Type III Procollagen Peptide (PIIINP) level showed significant difference among control and patients' groups ( $p < 0.001$ ) in such a way that the level was highest in patients with grade 3-4 liver fibrosis which was higher than both control group and patients with liver fibrosis of grade 0-1; but, there was no significant difference in its level between control group and patients with liver fibrosis of grade 0-1 ( $p > 0.05$ ). Receiver operator characteristic (ROC) analysis was carried out in order to evaluate the diagnostic potential of Serum collagen IV and Type III Procollagen Peptide (PIIINP) in the detection of liver fibrosis and the results were shown in figures 1 and 2 and table 4. In terms of sensitivity, the most sensitive among markers was Collagen IV followed by PIIINP. In terms of specificity, the most specific among markers was PIIINP followed by collagen IV. In terms of accuracy, the most accurate marker was Collagen IV followed by PIIINP. Receiver operator characteristic (ROC) analysis was carried out in order to evaluate The discriminative potential of Serum collagen IV and Type III Procollagen Peptide (PIIINP) in the detection of grade of liver fibrosis (grade 3-4 versus grade 0-1) and the results were shown in figures 3 and 4 and table 5. In terms of sensitivity, the most sensitive among markers was Collagen IV followed by PIIINP. In terms of specificity, the most specific among markers was PIIINP followed by Collagen IV. In terms of accuracy, the most accurate marker was PIIINP followed by Collagen IV.

**Conclusion:** Both serum type IV collagen and serum procollagen type III (PIIINP) are markers of detecting and grading liver fibrosis in patients with chronic liver disease with clinically acceptable levels of accuracy, sensitivity and specificity and can replace liver biopsy when it is contraindicated.

**Keywords:** IV collagen, procollagen type III (PIIINP), liver fibrosis

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## INTRODUCTION

The "gold standard" for the identification of liver disorders is liver biopsy. However, a liver biopsy is an intrusive operation that carries a risk of complications, and its sensitivity in detecting cirrhosis is not 100% accurate. Sampling mistake is also a fairly typical occurrence (Jin, 2007; Gudowska et al., 2017). In order to diagnose liver fibrosis and cirrhosis, non-invasive laboratory tests would be helpful (Lurie et al., 2015; Jarcuska et al., 2016). Extracellular matrix (ECM) formation in hepatic stellate cells (HSCs) is thought to be the primary source of the liver fibrosis process (Liu et al., 1997). Liver damage results from an increase in ECM deposits (about six times greater) in the Disse's space (Bataller and Brenner, 2005; Sarah et al., 2013).

Collagen makes up the majority of the ECM (Frantz et al., 2010; Yue, 2014). Type III collagen predominates in the liver (Colli et al., 2005; Baranova et al., 2011). The N-terminal propeptide of procollagen type III (PIIINP) separates from procollagen type III during the production of type III collagen, causing fibrogenesis to cause the release of ECM fragments into the blood. Since collagen is synthesized and deposited in the extracellular space, the amount of propeptide of procollagen type III can be used as a direct indicator of this (Cross et al., 2010). Alcoholic liver disease, viral hepatitis B and C, iron or copper excess, and autoimmune conditions are the most frequent causes of fibrosis (Gudowska et al., 2017). Although PIIINP is not a marker specific to the liver, a rise in propeptide of type III procollagen may signify the conversion of normal liver tissue into connective tissue. Other clinical illnesses such as congestive heart failure, hypertension, and coronary heart disease were also found to have higher quantities of PIIINP. These conditions are all linked to aberrant collagen formation. It most likely results from persistent inflammation (Gudowska et al., 2017).

Type IV collagen measured in serum has also been considered as a non-invasive tool in the assessment of liver fibrosis in patients with chronic liver disease and the results were promising (Stefano et al., 2021). The current study was aiming at evaluation of the role of serum type IV collagen and serum PIIINP as biomarkers for the detection and staging of liver fibrosis in a sample of Iraqi patients with chronic liver disease.

## PATIENTS AND METHODS

The current case control study included 100 patients with liver fibrosis and 50 healthy control individuals of comparable age and gender. The age range of patients was from 32 to 67 years. Patients were having chronic liver disease and they were grouped into 22, 36, 19 and 23 as having alcoholic liver disease, non-alcoholic liver disease, HBV and HCV. Demographic characteristics were obtained including age, gender and body mass index. Assessment of the following biochemical testes was performed to all

participants by ELISA: ALT, AST, ALP, GGT, TSB, Albumin, type IV collagen and PIIINP.

The study was done in Alimamain Alkadhimain medical city in Baghdad, Iraq during the period from April 2021 through April 2022. The study was approved by the ethical committee for research approval of the college of medicine/ Al-Nahrain university and verbal consent was obtained from all participants.

The data were transformed into and SPSS (statistical package for social sciences) software spread sheet (Chicago, IBM, USA, version 16) for purpose of statistical analysis. One way ANOVA was used to compare means among study groups followed by post hoc multiple comparison test. Chi-square test was used to compare proportions. Receiver operating characteristic (ROC) curve analysis was performed to finding the cutoff values. Statistical significance was suggested at  $p \leq 0.05$ .

## RESULTS

The demographic characteristics of patients and control subjects are demonstrated in table 1. Biochemical characteristics of liver function test of patients and control subjects are shown in table 2. Serum collagen IV and Type III Procollagen Peptide (PIIINP) of patients and control subjects are shown in table 3. There was significant difference in mean collagen IV among patients' and control groups ( $p < 0.001$ ) in such a way that highest level was reported in patients with grade 3-4 liver fibrosis and this level was significantly higher than that of patients with liver fibrosis grade 0-1 and control group ( $p < 0.05$ ) and the level of patients with liver fibrosis grade 0-1 was significantly higher than that of control group ( $p < 0.05$ ). Type III Procollagen Peptide (PIIINP) level showed significant difference among control and patients' groups ( $p < 0.001$ ) in such a way that the level was highest in patients with grade 3-4 liver fibrosis which was higher than both control group and patients with liver fibrosis of grade 0-1; but, there was no significant difference in its level between control group and patients with liver fibrosis of grade 0-1 ( $p > 0.05$ ).

Receiver operator characteristic (ROC) analysis was carried out in order to evaluate the diagnostic potential of Serum collagen IV and Type III Procollagen Peptide (PIIINP) in the detection of liver fibrosis and the results were shown in figures 1 and 2 and table 4. In terms of sensitivity, the most sensitive among markers was Collagen IV followed by PIIINP. In terms of specificity, the most specific among markers was PIIINP followed by collagen IV. In terms of accuracy, the most accurate marker was Collagen IV followed by PIIINP.

Receiver operator characteristic (ROC) analysis was carried out in order to evaluate The discriminative potential of Serum collagen IV and Type III Procollagen Peptide (PIIINP) in the detection of grade of liver fibrosis (grade 3-4 versus grade 0-1) and the results were shown in figures 3 and 4 and table 5. In terms of sensitivity, the most sensitive among markers was

Collagen IV followed by PIIINP. In terms of specificity, the most specific among markers was PIIINP followed by

Collagen IV. In terms of accuracy, the most accurate marker was PIIINP followed by Collagen IV.

**Table 1:** Demographic characteristics of patients and control subjects

<i>Characteristic</i>	<i>Control</i> <i>n = 50</i>	<i>Fibrosis (0-1)</i> <i>n = 50</i>	<i>Fibrosis (3-4)</i> <i>n = 50</i>	<i>P value</i>
Age (years)				
Mean ±SD	46.34 ±9.73	50.28 ±9.05	48.44 ±8.77	0.104 O
	A	A	A	NS
Range	31 -66	32 -67	33 -67	
BMI (kg/m <sup>2</sup> )				
Mean ±SD	24.92 ±3.37	34.19 ±6.21	33.86 ±5.84	< 0.001 O **
	B	A	A	
Range	18.8 -28.5	18.8 -44	25.5 -45.1	
Gender				
Male, n (%)	28 (56.0 %)	27 (54.0 %)	26 (52.0 %)	0.923 C
Female, n (%)	22 (44.0 %)	23 (46.0 %)	24 (48.0 %)	NS

n: number of cases; SD: standard deviation; BMI: body mass index; O: one way ANOVA; NS: not significant; \*\*: significant at  $p \leq 0.01$ ; Capital letters (A, B and C) were used to indicate the significance level following post hoc LSD multiple comparison so that similar letters indicate no significant difference whereas, different letters indicate significant difference at  $p \leq 0.05$

**Table 2:** Biochemical characteristics of liver function test of patients and control subjects

<i>Characteristic</i>	<i>Control</i> <i>n = 50</i>	<i>Fibrosis (0-1)</i> <i>n = 50</i>	<i>Fibrosis (3-4)</i> <i>n = 50</i>	<i>P value</i>
ALT (IU/L)				
Mean ±SD	12.66 ±3.76	92.48 ±6.80	92.56 ±7.73	< 0.001 O **
	B	A	A	
Range	6 -20	80 -105	80 -109	
AST (IU/L)				
Mean ±SD	12.42 ±3.41	93.90 ±14.77	89.66 ±9.98	< 0.001 O **
	C	A	B	
Range	8 -22	71 -144	70 -110	
ALP (IU/L)				
Mean ±SD	80.76 ±12.75	89.60 ±12.88	83.04 ±11.33	< 0.001 O **
	B	A	B	
Range	66 -120	66 -130	60 -100	
GGT (U/L)				
Mean ±SD	10.96 ±3.05	39.88 ±26.74	36.10 ±23.77	< 0.001 O **
	B	A	A	
Range	6 -19	20 -90	21 -99	
TSB (mg/dl)				
Mean ±SD	0.68 ±0.12	1.51 ±0.21	1.56 ±0.41	< 0.001 O **
	B	A	A	

Range	0.3 -0.8	1.2 -2	1.2 -2.6	
Direct bilirubin (mg/dl)				
Mean ±SD	0.29 ±0.08	0.81 ±0.19	0.95 ±0.39	< 0.001 O **
	C	B	A	
Range	0.2 -0.6	0.5 -1.2	0.5 -1.6	
Indirect bilirubin (mg/dl)				
Mean ±SD	0.40 ±0.07	0.70 ±0.12	0.68 ±0.13	< 0.001 O **
	B	A	A	
Range	0.2 -0.5	0.5 -1.1	0.49 -1	
Albumin (g/dl)				
Mean ±SD	41.70 ±1.61	38.56 ±1.61	35.81 ±0.76	< 0.001 O **
	A	B	C	
Range	39 -45	36 -42	35 -37	

n: number of cases; SD: standard deviation; ALT: alanine transaminase; AST: aspartate transaminase; TSB: total serum bilirubin; ALP: alkaline phosphatase; Albumin; GGT: gamma glutamyl transferase; O: one way ANOVA; NS: not significant; \*\*: significant at  $p \leq 0.01$ ; Capital letters (A, B and C) were used to indicate the significance level following post hoc LSD multiple comparison so that similar letters indicate no significant difference whereas, different letters indicate significant difference at  $p \leq 0.05$

**Table 3:** Serum laminin, hyaluronic acid, collagen IV and Type III Procollagen Peptide (PIIINP) of patients and control subjects

<i>Characteristic</i>	<i>Control</i> <i>n = 50</i>	<i>Fibrosis (0-1)</i> <i>n = 50</i>	<i>Fibrosis (3-4)</i> <i>n = 50</i>	<i>P value</i>
<b>Collagen IV (ng/ml)</b>				
<b>Mean ±SD</b>	263.88 ±145.67	314.76 ±145.94	542.38 ±50.49	< 0.001 O **
	C	B	A	
<b>Range</b>	133 -1113	120 -575	420 -664	
<b>PIIINP</b>				
<b>Mean ±SD</b>	290.24 ±89.33	246.76 ±129.62	671.80 ±204.28	< 0.001 O **
	B	B	A	
<b>Range</b>	100 -419	106 -750	260 -955	

n: number of cases; SD: standard deviation; PIIINP: Type III Procollagen Peptide (PIIINP); O: one way ANOVA; NS: not significant; \*\*: significant at  $p \leq 0.01$ ; Capital letters (A, B and C) were used to indicate the significance level following post hoc LSD multiple comparison so that similar letters indicate no significant difference whereas, different letters indicate significant difference at  $p \leq 0.05$

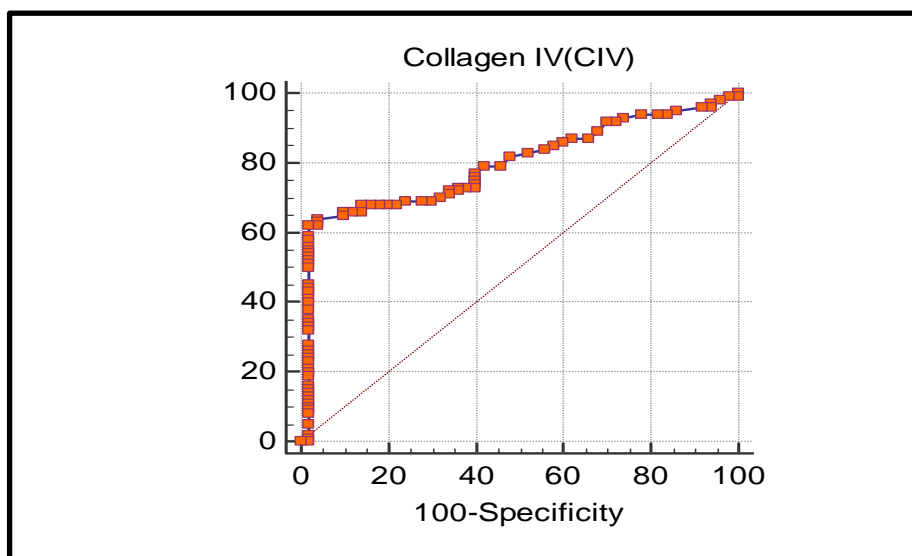


Fig.1: Receiver operator characteristic (ROC) curve analysis to calculate the cutoff value of collagen IV that can predict a diagnosis of liver fibrosis in terms of sensitivity and specificity

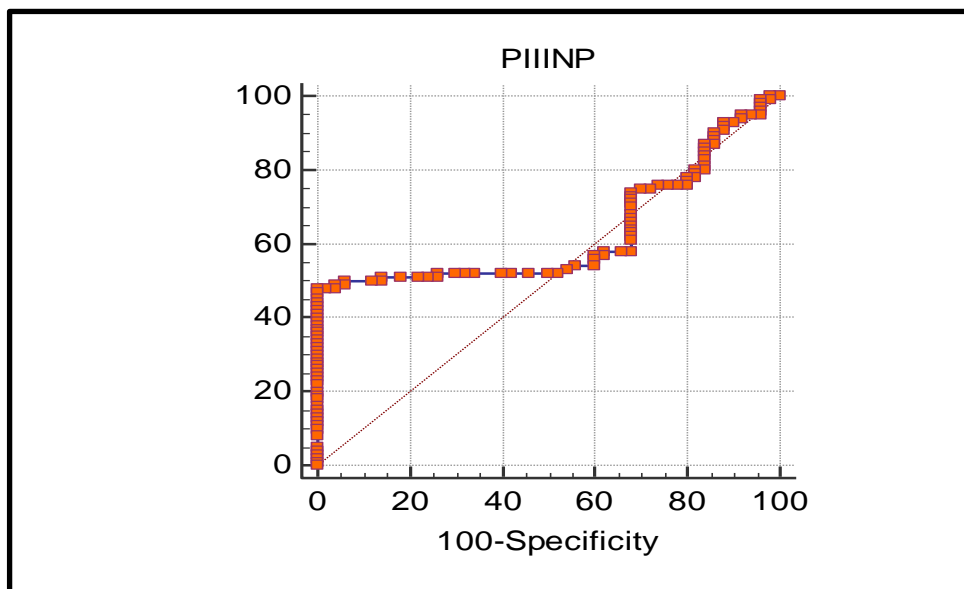


Fig.2: Receiver operator characteristic (ROC) curve analysis to calculate the cutoff value of collagen IV that can predict a diagnosis of liver fibrosis in terms of sensitivity and specificity

**Table 4:** The parameters of receiver operator characteristic (ROC) curve analysis for diagnosis of liver fibrosis

<i>Characteristic</i>	<i>Collagen IV</i>	<i>PIIINP</i>
<b>Cutoff</b>	>413	>419
<b>AUC</b>	0.795	0.627
<b>95 % CI</b>	0.722 to 0.857	0.545 to 0.705
<b>p-value</b>	< 0.001**	0.005**
<b>Sensitivity %</b>	64.0	48.0
<b>Specificity %</b>	96.0	100.0
<b>Accuracy %</b>	79.5	62.7

AUC: area under the curve; CI: confidence interval; \*\*: significant at  $p \leq 0.01$

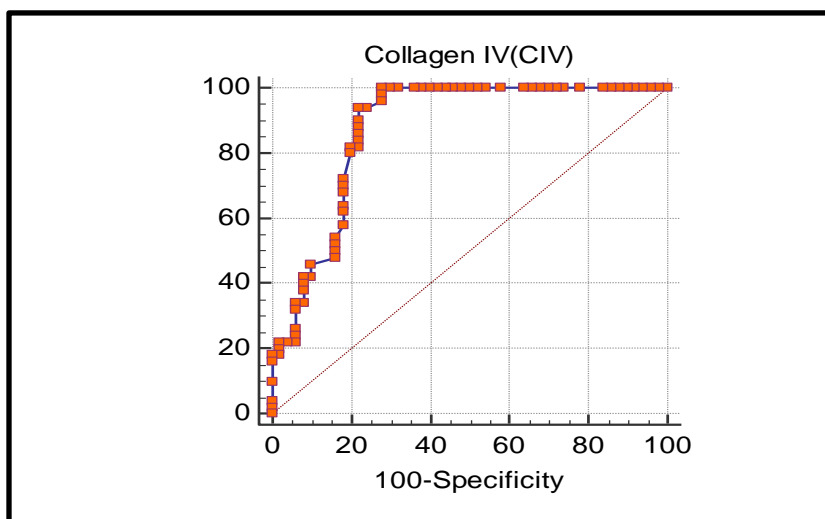


Fig.3: Receiver operator characteristic (ROC) curve analysis to calculate the cutoff value of collagen IV that can discriminate between grade 3-4 liver fibrosis and grade 0-1 liver fibrosis

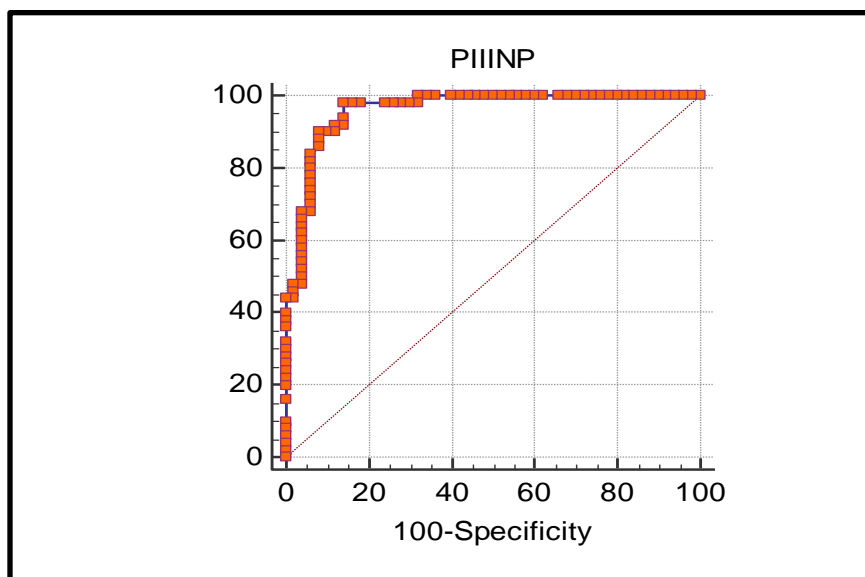


Fig.4: Receiver operator characteristic (ROC) curve analysis to calculate the cutoff value of PIIINP that can discriminate between grade 3-4 liver fibrosis and grade 0-1 liver fibrosis

**Table 5:** The parameters of receiver operator characteristic (ROC) curve analysis for discrimination among grades of liver fibrosis

<i>Characteristic</i>	<i>Collagen IV</i>	<i>PIIINP</i>
<b>Cutoff</b>	>413	>305
<b>AUC</b>	0.875	0.96
<b>95 % CI</b>	0.794 to 0.933	0.900 to 0.989
<b>p-value</b>	< 0.001	< 0.001
<b>Sensitivity %</b>	100.0	98.0
<b>Specificity %</b>	72.0	86.0
<b>Accuracy %</b>	87.5	96.0

AUC: area under the curve; CI: confidence interval; \*\*: significant at  $p \leq 0.01$

## DISCUSSION

With respect to serum collagen IV and type III procollagen Peptide (PIIINP) in present study, there significant correlation with grade of liver fibrosis in such a way that highest level was reported in patients with grade 3-4 fibrosis followed by patients with grade 0-1 fibrosis and lastly by control group. In present study also, were able to identify, the following cutoff values >413 and >419 for hyaluronic acid, laminin, collagen IV and Type III Procollagen Peptide (PIIINP) in the detection of liver fibrosis. All these cutoff values showed excellent level of specificity, but with variable levels of sensitivity. In addition, were able to find the following cutoff values >413 and >305 for serum collagen IV and type III procollagen peptide (PIIINP) for the discrimination between grade 3-4 liver fibrosis and grade 0-1 liver fibrosis. Collagen IV was the most sensitive marker.

Hirayama et al. (1996) in their study showed that serum type IV collagen increases with liver fibrosis and can be regarded as a marker of liver fibrosis, in addition, they show that serum type IV collagen levels had direct correlation with severity of liver fibrosis so these findings supports present study findings in the present study with respect to the potential of serum type IV collagen in detecting and grading severity of liver fibrosis; however, they showed that serum type III procollagen peptide (PIIINP) increases with all types of chronic liver disease and cannot discriminate the grade of liver fibrosis in clear contradiction to present study findings.

According to Santos et al (2005), patients with liver fibrosis presented significantly higher mean type IV collagen values than those with no liver fibrosis, and there were significant correlation between this parameter and the stage of fibrosis in the biopsy and they found type IV collagen was poor predictor of fibrosis.

## CONCLUSION

Both serum type IV collagen and type III procollagen peptide (PIIINP) are markers of detecting and grading liver fibrosis in patients with chronic liver disease with clinically acceptable levels of accuracy, sensitivity and specificity and can replace liver biopsy when it is contraindicated.

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