The Role of Serum Laminin and Serum Hyaluronic Acid 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 laminin and serum hyaluronic acid 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, hyaluronic acid and laminin. Results: There was significant difference in mean Hyaluronic acid 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 ($p < 0.05$). There was also significant difference in mean laminin 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 ($p < 0.05$). Receiver operator characteristic (ROC) analysis was carried out in order to evaluate the diagnostic potential of Serum laminin and hyaluronic acid, collagen IV. In terms of sensitivity, the most sensitive among markers was Hyaluronic acid followed by Laminin. Receiver operator characteristic (ROC) analysis was carried out in order to evaluate the discriminative potential of Serum laminin and hyaluronic acid in the detection of grade of liver fibrosis (grade 3-4 versus grade 0-1). In terms of sensitivity, the most sensitive among markers was Hyaluronic acid followed by Laminin. In terms of specificity, the most specific among markers was Hyaluronic acid followed by Laminin. In terms of accuracy, the most accurate marker was Hyaluronic acid then followed by Laminin. Conclusion: Both serum laminin and serum hyaluronic acid 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.

1. Introduction

The process of liver fibrosis involves the formation and deposition of numerous extracellular matrix constituents (ECM). Noncollagenous glycoproteins such laminin, hyaluronic acid (HA), and proteoglycans are a few of these components (Parsian et al., 2010). For determining the rate at which liver fibrosis is progressing, choosing a treatment, and keeping track of the condition, staging the condition and assessing the degree of inflammation are helpful (Suk and Kim, 2015; Stasi and Milani, 2017). The standard diagnostic for grading and staging hepatic fibrosis and inflammation is still a liver biopsy. However, noninvasive techniques are now favoured due to several limitations of the liver biopsy (Stasi and Milani, 2017). In order to measure the degree of liver fibrosis in chronic liver disorders, some research suggested using HA and LN as indicators (Parsian et al., 2010). A glycosaminoglycan found in the extracellular spaces is hyaluronic acid. The sinusoidal endothelial cells in the liver mostly manufacture HA while the hepatic stellate cells primarily breakdown it. Patients with cirrhosis and chronic hepatitis C showed an increase in serum HA levels (Halfon et al., 2005; Parsian et al., 2010).

Hepatocytes and sinusoidal cells produce laminin, one

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of the primary glycoproteins of the basement membrane (Rosa and Parise, 2008). The majority of serum laminin is produced by lipocytes or stellate cells. In advanced fibrosis phases, higher serum laminin levels were seen (Younesi et al., 2019). The adoption of a non-invasive instrument to detect and assess liver fibrosis in patients with chronic liver disease is urgently necessary because histological examination of liver fibrosis may be challenging due to contraindications. The aim of the current study was to evaluate the role of serum laminin and serum hyaluronic acid as biomarkers for the detection and staging of liver fibrosis in a sample of Iraqi patients with chronic liver disease.

2. 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, hyaluronic acid and laminin. The study was done in Alimamain Alkadhimain medical city in Baghdad, Iraq during the period from April 2021 through Aril 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 tto finding the cutoff values. Statistical significance was suggested at $p \leq 0.05$.

3. Results

The demographic characteristics of patients and control subjects are demonstrated in table 1. There was no significant difference in mean age and gender proportions among study groups ($p>0.05$); however, patients with liver fibrosis had significantly higher mean BMI in comparison with control group ($p < 0.001$); 34.19 ±6.21 kg/m2 versus 33.86 ±5.84 kg/m2 versus 24.92 ±3.37 kg/m2, respectively. Biochemical characteristics of liver function test of patients and control subjects are shown in table 2. Significant variation has been observed in all these characteristics among study groups ($p < 0.001$). Serum laminin and hyaluronic acid of patients and control subjects are shown in table 3. There was significant difference in mean Hyaluronic acid 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 ($p < 0.05$). There was also significant difference in mean laminin 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 ($p < 0.05$).

Receiver operator characteristic (ROC) analysis was carried out in order to evaluate the diagnostic potential of Serum laminin and hyaluronic acid 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 Hyaluronic acid followed by Laminin. In terms of specificity, the most specific among markers was laminin followed by hyaluronic acid. In terms of accuracy, the most accurate marker was Hyaluronic acid followed by Laminin. Receiver operator characteristic (ROC) analysis was carried out in order to evaluate the discriminative potential of Serum laminin and hyaluronic acid 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 Hyaluronic acid followed by Laminin. In terms of specificity, the most specific among markers was Hyaluronic acid followed by Laminin. In terms of accuracy, the most accurate marker was Hyaluronic acid then followed by Laminin.

Table 1: Demographic characteristics of patients and control subjects

Characteristic	Control n = 50	Fibrosis (0-1) n = 50	Fibrosis (3-4) n = 50	P value
Age (years)				
Mean ±SD	46.34 ±9.73 A	50.28 ±9.05 A	48.44 ±8.77 A	0.104 O NS
Range	31 -66	32 -67	33 -67	
BMI (kg/m2)				
Mean ±SD	24.92 ±3.37 B	34.19 ±6.21 A	33.86 ±5.84 A	< 0.001 O **
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 NS
Female, n (%)	22 (44.0 %)	23 (46.0 %)	24 (48.0 %)	

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				
Characteristic	Control n = 50	Fibrosis (0-1) n = 50	Fibrosis (3-4) n = 50	P value
ALT (IU/L)				
Mean ±SD	12.66 ±3.76 B	92.48 ±6.80 A	92.56 ±7.73 A	< 0.001 O **
Range	6 -20	80 -105	80 -109	
AST (IU/L)				
Mean ±SD	12.42 ±3.41 C	93.90 ±14.77 A	89.66 ±9.98 B	< 0.001 O **
Range	8 -22	71 -144	70 -110	
ALP (IU/L)				
Mean ±SD	80.76 ±12.75 B	89.60 ±12.88 A	83.04 ±11.33 B	< 0.001 O **
Range	66 -120	66 -130	60 -100	
GGT (U/L)				
Mean ±SD	10.96 ±3.05 B	39.88 ±26.74 A	36.10 ±23.77 A	< 0.001 O **
Range	6 -19	20 -90	21 -99	
TSB (mg/dl)				
Mean ±SD	0.68 ±0.12 B	1.51 ±0.21 A	1.56 ±0.41 A	< 0.001 O **
Range	0.3 -0.8	1.2 -2	1.2 -2.6	
Direct bilirubin (mg/dl)				
Mean ±SD	0.29 ±0.08 C	0.81 ±0.19 B	0.95 ±0.39 A	< 0.001 O **
Range	0.2 -0.6	0.5 -1.2	0.5 -1.6	
Indirect bilirubin (mg/dl)				
Mean ±SD	0.40 ±0.07 B	0.70 ±0.12 A	0.68 ±0.13 A	< 0.001 O **
Range	0.2 -0.5	0.5 -1.1	0.49 -1	
Albumin (g/dl)				
Mean ±SD	41.70 ±1.61 A	38.56 ±1.61 B	35.81 ±0.76 C	< 0.001 O **
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 ≤ 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 ≤ 0.05				

Table 3: Serum laminin, hyaluronic acid, collagen IV and Type III Procollagen Peptide (PIIINP) of patients and control subjects				
Characteristic	Control n = 50	Fibrosis (0-1) n = 50	Fibrosis (3-4) n = 50	P value
Hyaluronic acid (ng/ml)				
Mean ±SD	58.48 ±12.18 C	85.70 ±8.31 B	114.90 ±27.06 A	< 0.001 O **
Range	24 -86	65 -97	11 -187	
Laminin (pg/ml)				
Mean ±SD	753.16 ±173.92 C	1098.80 ±294.08 B	1774.30 ±453.49 A	< 0.001 O **
Range	44 -960	429 -1980	864 -2732	
n: number of cases; SD: standard deviation; PIIINP: Type III Procollagen Peptide (PIIINP); O: one way ANOVA; NS: not significant; **: significant at p ≤ 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 ≤ 0.05				

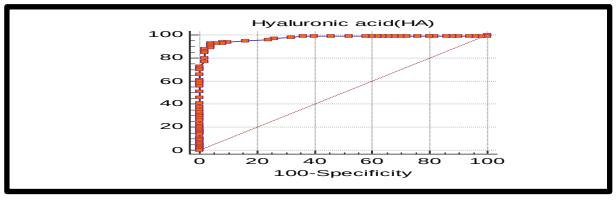

Figure 1: Receiver operatory characteristic (ROC) curve analysis to calculate the cutoff value of hyaluronic acid that can predict a diagnosis of liver fibrosis in terms of sensitivity and specificity

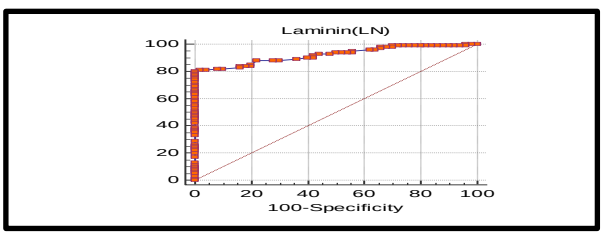

Figure 2: Receiver operatory characteristic (ROC) curve analysis to calculate the cutoff value of laminin that can predict a diagnosis of liver fibrosis in terms of sensitivity and specificity

Table 4: The parameters of receiver operator characteristic (ROC) curve analysis for diagnosis of liver fibrosis		
Characteristic	Hyaluronic acid	Laminin
Cutoff	>75	>960
AUC	0.973	0.922
95 % CI	0.932 to 0.992	0.867 to 0.960
p-value	< 0.001**	< 0.001**
Sensitivity %	93.0	80.0
Specificity %	96.0	100.0
Accuracy %	97.3	92.2
AUC: area under the curve; CI: confidence interval; **: significant at p ≤ 0.01		

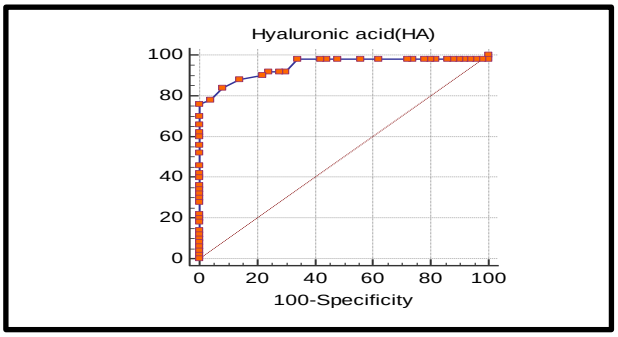

Figure 3: Receiver operatory characteristic (ROC) curve analysis to calculate the cutoff value of hyaluronic acid that can discriminate between grades 3-4 liver fibrosis and grade 0-1 liver fibrosis

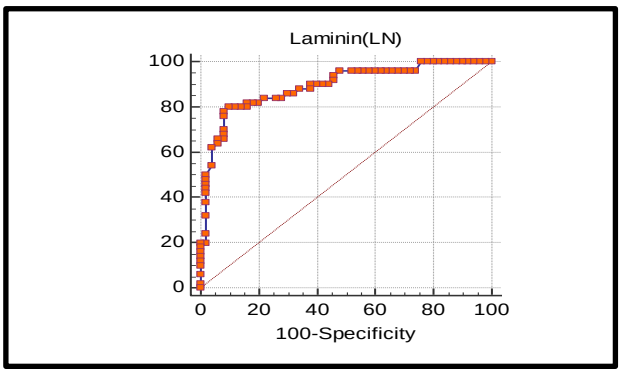

Figure 4: Receiver operatory characteristic (ROC) curve analysis to calculate the cutoff value of laminin that can discriminate between grades 3-4 liver fibrosis and grade 0-1 liver fibrosis

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

Characteristic	Hyaluronic acid	Laminin
Cutoff	>95	>1414
AUC	0.944	0.891
95 % CI	0.879 to 0.980	0.813 to 0.945
p-value	< 0.001	< 0.001
Sensitivity %	84.0	80.0
Specificity %	92.0	90.0
Accuracy %	94.4	89.1

AUC: area under the curve; CI: confidence interval; **: significant at p ≤ 0.01

4. Discussion

With respect to serum hyaluronic acid and serum laminin 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, >75 and >960 for hyaluronic acid and laminin in the detection of liver fibrosis. These cutoff values showed excellent level of specificity, but with variable levels of sensitivity, the highest was associated with serum hyaluronic acid.

In addition, were able to find the following cutoff values, >95 and >1414, for serum hyaluronic acid and laminin for the discrimination between grade 3-4 liver fibrosis and grade 0-1 liver fibrosis. Hyaluronic acid was the most specific marker.

Previous studies demonstrated that laminin is a fibrosis biomarker in alcoholic liver disease, viral hepatitis, and NAFLD. In particular, high laminin levels were shown to be associated with the severity of fibrosis and hepatitis (Hirayama et al., 1996; Abeer et al., 2013; Younesi et al.; 2019). (Hirayama et al., 1996) in their study showed that serum laminin increases with liver fibrosis and can be regarded as a marker of liver fibrosis, in addition, they show that serum laminin level 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 laminin in detecting and grading severity of liver fibrosis.

Younesi et al in 2019, compared the diagnostic accuracy of serum levels of laminin and hyaluronic acid in detecting liver fibrosis, and found that the accuracy of laminin was superior to that of hyaluronic acid, 0.89, 0.82, respectively; therefore, current study agrees with Younesi et al with respect to the diagnostic potential of laminin and hyaluronic acid, but, present study disagrees in that in current study results hyaluronic acid was superior in terms of accuracy to laminin.

According to the studies performed on HBV patients, laminin can be a clinically beneficial serum marker for estimating significant fibrosis, particularly when liver biopsy is contraindicated. The studies have shown that elevated laminin serum levels as ECM components indicate a chronic liver injury consequence, which leads to architectural changes in the liver parenchyma and eventually liver fibrosis (Parsian et al., 2010; Lia et al., 2012; Abeer et al., 2013). The most elevated serum laminin levels were found in the cirrhotic group and the cutoff point of 60.9 ng/mL showed a sensitivity and specificity of 71.4% and 77.1%, respectively, in the assessment of significant fibrosis. Additionally, it was reported by Tawhida et al (2010) that serum laminin concentration could be useful in decreasing the need for liver biopsy, but not to replace liver biopsy. Other researchers have reported that a serum cutoff value of 52.0 ng laminin/mL gives a reliable sensitivity, positive predictive value, and negative predictive value for discriminating the various liver fibrosis stages. A significant positive correlation was also observed between serum laminin level and liver fibrosis stages (Parsian et al., 2011; Saad, 2014). Other results indicated that the serum laminin concentration significantly increased with the level of hepatic fibrosis (Parsian et al., 2010; Lia et al., 2012; Gressner AM, Tittor, 1986). Parsian et al (2010) reported that in HBV patients, the development of inflammation and liver fibrosis would be accompanied by the impairment of liver endothelial cell function and a decrease in laminin degradation, eventually leading to an increase in serum laminin level.

El-Mezayen et al (2015) indicated that the serum laminin level increased in accordance with liver

fibrosis stages, and the AUC of 0.71 was obtained for the discrimination of patients with severe liver fibrosis from those with mild fibrosis. Therefore, the serum laminin level could be considered a reliable and additional non-invasive tool for the evaluation of liver fibrosis.

According to Abeer et al (2013), serum laminin at value 107.5ng/ml was the optimal cut-off value for diagnosis of significant fibrosis. According to Santos et al (2005), patients with liver fibrosis presented significantly higher mean laminin, and hyaluronan, values than those with no liver fibrosis, and there were significant correlation between these parameters and the stage of fibrosis in the biopsy and they found that laminin values >282 ng/ml were those with the best diagnostic performance, with 87% accuracy, but type IV collagen was poor predictor of fibrosis and they concluded that laminin values presented a better diagnostic accuracy than hyaluronan, and type IV collagen.

5. Conclusion

Both serum laminin and serum hyaluronic acid 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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