Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 9695 - 9700
Received 25 April 2021; Accepted 08 May 2021.

Assessment of Non-Invasive Biochemical Markers as a Predictive of Liver Fibrosis in Patients with Chronic Hepatitis B

Mostafa Salman Hasan[1],Dawood Salman Mahdi[1] and Hamid Jaddoa Abbas[2]

(1) Southern Technical University, Health and Medical technology College.Iraq, Basra
(2) Al-Faiha'a Teaching Hospital, Iraq, Basra.
Corresponding: dr.dawds@stu.edu.iq.

Abstract

Background: Liver fibrosis is a complex fibrogenic and inflammatory processesthat results fromchronic liver injury and represents an early step in the progression of liver cirrhosis(Trivella *et al*., 2020).Generally liver fibrosis considered wound-healing process aimed at maintaining organ integrity and this development causes increase of laminin, collagen type III, collagen type IV and hyalouronate, these markers consider the main of extracellular matrix (Lambrecht *et al*., 2019).

The aim of study: To study the fibrosis of liver in chronic hepatitis B patients by noninvasive direct and indirect biomarkers and evaluation the diagnostic efficiency of these biomarkers.

Materials andMethods: A case control study, involves 119 participants:50 healthy persons as control and 69 patients with chronic hepatitis B, they were diagnosed by PCR technique. The direct biomarkers (serum laminin, collagen type IV, N-terminal peptide III collagen PIIINP, hyaluronate) and indirect markers (serum total bilirubin, direct bilirubin,aspartate aminotransferaseAST,alanine aminotransferaseALT, alkaline phosphataseALP, albumin and gamma glutamyltransferase(GGT), in addition to cholesterol were estimation by standard methods.

Results: Comparison between control and HBV patients showed a significant difference (P.value<0.05) for liver function tests and fibrotic marker:laminin(33.12±5.65 control vs. 59.80±21.2 HBV),PIIINP(6.48±2.42 control vs.20.55±11.97 HBV),type IV collagen(18.97±10.04 control vs. 84.42±80.83 HBV),hyaluronate (32.68±15.29 control vs. 152.26±148.55 HBV).The sensitivity of fibrotic markers: laminin,PIIINP, type IV collagen and hyaluronateweremore than 80% when used the ROC curve.

Conclusions: estimation of noninvasive biochemical markers could be detected fibrosis of liver in hepatitis B with significant accuracy. These biomarkers may use as alternative to liver biopsy and contributed to assessing the progression and treatment of liver fibrosis.

Key word:Hepatitis B, liver fibrosis,Liver Biopsy.

Introduction:

The liver is one of the largest organsin the human body. It is comprised of different types of cells that are responsible for many functions, such as metabolism, immunity, digestion, detoxification and storage*(*Jophlin *et al.,* 2018). Viral hepatitis infection isbecoming a significant danger to human healthduring the last decade, about 1.3 million people die annually from infection with viral hepatitis worldwide.These deaths arepredominantly associated with cirrhosis and hepatocellularcarcinoma, resulting fromchronic infections with hepatitis B virus (HBV:887000 deaths), (Shrivastava2019).

Materials and methods

A case control study, involves 119 participants; 50 healthy control(26 males and 24 females) and 69 patients (36 males and 33 females) with chronic hepatitis type B for at least 6 months or more without treatment.The patientswere attended toAl-Faiha'a Gastrointestinal Center, Basra, Iraq. The patients were diagnosed by full clinical examinations and laboratory

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 9695 - 9700
Received 25 April 2021; Accepted 08 May 2021.

investigations..ThePatients,who had chronic diseases were excluded from the study unless the patients in our study.The parameters laminin, P3NP, type IV collagen and hyaluronatewere measurement by chemiluminescent immunoassay (CLIA), Mindray CL1000 auto analyzer. Liver function testsand cholesterol measurement by Cobas INTEGRA plus 400 auto-analyzer.

Statistical analysis

The results wereanalyzed by IBM SPSS Statistics 22.0 programand p-values ≤0.05 was considered as statistically significant.

Results:

A- Comparison between control and hepatitis B patients

This study was shown, that all the variables were shown significantly differencesbetween control and hepatitis B patients, except in age , sex as well as cholesterol (P values <0.05),(table 1).

Table 1: Comparison between control and hepatitis B patients

Variables		Control (N= 50)	Hepatitis B (N= 69)	P.Value
Age		42.2 ± 15.7	43.0 ± 15.9	0.784
Sex	**Male**	26 (52.0%)	36 (52.2%)	0.912
	Female	24 (48.0%)	33 (47.8%)	
	Total	50 (100%)	69 (100%)	
Bilirubin- T		0.41 ± 0.17	2.58 ± 2.27	0.0001
Bilirubin- D		0.14 ± 0.07	1.98 ± 1.86	0.0001
AST		16.24 ± 4.61	60.37 ± 56.11	0.0003
ALT		29.90 ± 10.26	74.73 ±71.5	0.0002
ALP		75.12 ± 15.09	115.4 ± 73.78	0.0001
GGT		17.65 ± 7.81	88.19 ± 83.06	0.0001
Albumin		4.28 ± 0.24	4.11 ± 0.51	0.001
Laminin		33.12 ± 5.65	59.80 ± 21.27	0.0001
Hyaluronate		32.68 ± 15.29	152.26± 148.55	0.001
P3NP		6.48 ± 2.42	20.55 ± 11.97	0.001
Type 4 collagen		18.97 ± 10.04	84.42 ± 80.83	0.001
Cholesterol		167.98 ± 27.34	162.55 ± 34.48	0.358

B- Comparison between control and hepatitis B patients according to gender

The comparison between control and hepatitis B patients according to gender was shown in (Table 2). The current study was observed that male patient's group with hepatitis B was significantly differ from the control group in all variables (P.values<0.05); except for age(P value > 0.05).On other hand, femalepatients'group differ from the control group in all variables (P. value <0.05); except ageand cholesterol (P. values >0.05).

Table2:Comparison between control and hepatitis B patientsaccording to gender

Variables	Males	Females

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 9695 - 9700
Received 25 April 2021; Accepted 08 May 2021.

	Control (N= 26)	Hep. B (N = 36)	P. Value	Control (N = 24)	Hep. B (N = 33)	P. Value
Bilirubin- T	0.47 ± 0.13	2.61 ± 2.32	0.0001	0.35 ± 0.18	2.55 ± 2.25	0.0001
Bilirubin- D	0.17 ± 0.08	1.77 ± 1.75	0.0001	0.10± 0.06	2.21 ± 2.17	0.0001
AST	17.98 ± 4.79	57.20 ±49.9	0.005	14.36 ± 3.65	63.81 ±62.7	0.007
ALT	32.80 ± 9.79	85.05 ± 83.9	0.0003	26.88± 10.06	63.48 ± 53.9	0.0045
ALP	74.07 ± 14.80	108.13 ±82	0.012	76.25 ± 16.85	123.49 ± 63	0.001
GGT	21.88 ± 8.08	73.13 ± 69.3	0.0003	13.06 ± 4.2	74.21 ± 67.8	0.001
Albumin	4.38 ± 0.18	4.23 ± 0.48	0.032	4.37 ± 0.3	3.99 ± 0.53	0.001
Laminin	33.08 ± 5.25	59.44±23.72	0.002	33.15 ± 6.16	63.14 ±39.3	0.001
Hyaluronate	32.11 ±12.66	124.46 ± 107.3	0.001	33.3 ± 17.98	139.01±112.29	0.001
P3NP	6.55± 1.45	22.95± 13.67	0.0001	6.41 ± 3.2	17.94 ± 8.84	0.003
Type 4 collagen	19.86 ±8.96	94.63± 101.00	0.0001	18 ± 11.2	73.27 ± 49.81	0.001
Cholesterol	176.15 ± 24.4	151.47± 30.85	0.003	159.12 ± 28	174.63 ± 34.0	0.067

C- Receiver-operating characteristic (ROC) curve analysisfor the diagnosis of hepatitis B

Receiver-operating characteristic (ROC) curve analysis for the diagnosis of liver fibrosis in hepatitis Bwas shown in (Table3).The results of this study were shownthat Laminin, hyaluronate, P3NP and Type4 collagen are of good diagnosis efficiency.

Table 3 Receiver-operating characteristic (ROC) curve analysis for the diagnosis of liver fibrosis in hepatitis B.

Variables	Area under the ROC curve	p-value (AUC0 =0.5)	Best cut-off criterion	Sensitivity (%)	Specificity (%)	Efficiency	PPV	NPV
Laminin	0.817	0.0001	33.65	82.6	48.0	82.2	61.2	72.7
Hyaluronate	0.858	0.0001	38.05	80.1	66.0	80.33	70.2	76.7
P3NP	0.668	0.002	5.15	80.0	32.0	80.16	54.1	61.5
Type4 collagen	0.880	0.001	22.25	81.2	70	81.35	73.0	78.7

PPV:positive predictive value,NPV: negative predictive value.

D- Identification of risk of liver fibrosis in patients with hepatitis B

The results of this study were revealed that there were no parameters had statistical significance (P. values >0.05). However, the magnitude of odds ratioswas varied. Laminin, collagen, GGT and AST were the best predictors, (Table 4).

Table 4: Identification of risk of liver fibrosis in patients with hepatitis B

Variables	P. Value	Odd ratio	95% Confidence Limits

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 9695 - 9700
Received 25 April 2021; Accepted 08 May 2021.

			Low	Upper
Bilirubin T	0.917	0.0001	0.0001	*
Bilirubin D	0.912	0.0001	0.0001	*
AST	0.966	11.781	0.0001	66.0
ALT	0.933	0.028	0.0001	1.790
ALP	0.967	0.545	0.0001	12.000
GGT	0.959	20.141	0.0001	3.400
Albumin	0.999	0.080	0.0001	*
Laminin	0.930	119.103	0.0001	1.700
Hyaluronate	0.939	5.041	0.0001	4.100
P3NP	0.965	0.0001	0.0001	1.810
Type4collagen	0.914	33.679	0.0001	2.110

*: the upper confidence limit was very low.

E- Correlations among the variables for all patients

The correlations among the variables for patients were shownin (Table5) .This study was considered correlation coefficient of 0.6 or more as significant indication of correlation between binary variables.The following pairs of variables were significant correlated:(total bilirubin and direct bilirubin),(ALT and AST),(ALP and P3NP), (Laminin and hyaluronate), (P3NP and type 4 collagen). All others variables were not significant.

Table 5:Correlations among the variables for all patients

	Bili. D	**AST**	**ALT**	**ALP**	**GGT**	Albumin	Laminin	Hyalur.	**P3NP**	**Type 4Coll.**
Bilirubin T	0.92	0.46	0.58	0.40	0.22	0.25	0.24	0.23	0.34	0.51
Bilirubin D	1	0.42	0.58	0.408	0.18	0.25	0.26	0.21	0.27	0.43
AST		1	0.63	0.38	0.40	0.10	0.54	0.34	0.43	0.57
ALT			1	0.35	0.315	0.10	0.31	0.21	0.43	0.47
ALP				1	0.52	0.40	0.58	0.58	0.65	0.56
GGT					1	0.36	0.46	0.54	0.56	0.48
Albumin						1	0.38	0.44	0.45	0.44
Laminin							1	0.65	0.57	0.57
Hyalur.								1	0.55	0.52
P3NP									1	0.72

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 9695 - 9700
Received 25 April 2021; Accepted 08 May 2021.

Type4Coll.										1

Discussion:

The hepatitis B virus is remaining the predominant cause of chronic liver disease and liver-related morbidity worldwide. This condition is considered to be the major risk factor for cirrhosis. The invasive nature of liver biopsy for diagnosing and grading of liver fibrosis and the possibility of sampling error,necessitate the availability of non-invasive methods for fibrosis assessment.

The assessment of liver fibrosis in hepatitis B patients by noninvasive biomarkersin thisstudy,wasshowed that thesebiomarker performed well to predicting liver fibrosis and cirrhosis. This result was agreement with(Elmdams *et al*., 2021)**.**

The current study revealedthat:(Laminin, hyaluronate, P3NP and type IV collagen) parameters in hepatitis B(59.80, 152.26, 20.55, 84.42) respectivelywere higher than control withsignificant differences when compared between hepatitis Bwith control, this results indicators of development hepatic fibrosis this results was agreement with (Wangensteen & Chang, 2021).

This results in present study may bedue to arises offibrogenesis in the liver from the activation of hepatic stellate cells after chronic liver injury by chronic viral infectionand activated stellate cell proliferates undergoes transformation to acquire the myofibroblastthis was agreement with(Ramírez-Fernández, 2020), the laminin deposition with collagensto formation of a true basement membrane along sinusoids, this study agreement with (Karsdal *et al.*, 2019).

The mean of lamnine and hyaluronate parameters in the femalepatients (63.14, 139.01) respectively higher than male patients (59.44, 124.46) this results may be due to effect of the estrogen hormone in femalesthat induce of the hepatic stellate cells and lead to production of ECM this results agreement with (Ezhilarasan, 2020).

The mean of the collagen type 4 and PIIINP in the male patients (94.63, 22.95) respectively higher than female patients (73.27, 17.94) , this results may be due to the collagen its synthesis in the skeletal muscles,the collagen in the male higher than female patients (Maren S, 2014).

The receiver operating characteristic (ROC) curve was used for diagnosis of liver fibrosis in hepatitis B,the resultswere revealed that the laminin, hyaluronate, P3NP and type4 collagen were hadROC more than 0.6, with high sensitivity (more than 80 %) for all parameters with specificity 48%,66%, 32% and 70%, respectively. These results were agreement with(Hu *et al.*, 2019). They reported that the Type IV collagen is a crucial component of hepatic ECM which is deposited integrally in matrix.

Serum estimation of type IV collagen is a sign of direct degradation and has positive correlation with grade of liver fibrosis. Combinatorial use of type IV collagen with PIIINP has a sensitivity and specificity of 88%.Increased concentrations in serum are attributable to increased production and decreased hepatic elimination or both.

Serum HA levels are related to stage of fibrosis and degree of necroinflammation. High levels have been detected in liver fibrosis with varied etiology. HA has sensitivity and specificity of 88–95% and 86–100%, respectively.Positive and negative predictive value of HA has been reported as 61% and 98–100%, respectively.

Serum laminin levels are elevated in liver fibrosis irrespective of etiology and have a correlation with severity of fibrosis and liver inflammation.In liver fibrosis, laminin increases around the vessels, in perisinusoidal space and portal triad.Laminin had sensitivity and specificity of 87 and 74%, respectively, with positive-predictive value of 77% and negative-predictive value of 85%.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 9695 - 9700
Received 25 April 2021; Accepted 08 May 2021.

Estimations of serum HA and laminin have good prognostic value for liver fibrosis complication. These tests can be used for non-invasivemonitoring of disease activity, and assessment of post-treatmentfibrosis and activity decrease in patients with chronic viral hepatitis.Fibrosis marker findings of this study could be used to contributefuture clinical studies that should be conducted in an effort todevelop a specific anti-fibrotic treatment.
The identify of risk liver fibrosis by multivariable logistic regression analysis,the results were shownthat some variables in the model were notsignificant(Pvalue>0.05),but the odd ratios in theseparameters are more riskcomparedwithothersparameters.This result is agreed with (Karsdal*et al.*, 2019).

The correlation among all parameters in present study shown the correlation between (bilirubin total with bilirubin direct), (ALT with AST), (ALP with P3NP), (laminin with Hyaluronate), (P3NP with Type 4 collagen).

Conclusions:

The direct and in direct non-invasive biochemical markers can be use as predictive of liver fibrosis, aswell as assessment the progression and treatment offibrosis in patients with chronic hepatitis B virus.

References:

1. Elmdams, M. R., Gad, S. A. B. & Gawish, M. S. F. (2021). A Novel Noninvasive Index for Assessment of Liver Fibrosis and Cirrhosis in Patients with Chronic Hepatitis C Virus Infection. The Egyptian Journal of Hospital Medicine, 82(1):156–163PP.
2. Ezhilarasan, D. (2020). Critical role of estrogen in the progression of chronic liver diseases. Hepatobiliary & Pancreatic Diseases International.19(5): 429-434 PP.
3. Hu, Zhu, Zhang, Chen;et al (2019). Potential circulating biomarkers of circulating chemokines CCL5, MIP-1β and HA as for early detection of cirrhosis related to chronic HBV (hepatitis B virus) infection. BMC Infectious Diseases, 19(1): 523 PP.
4. Jophlin, L. L., Koutalos, Y.; et al , (2018). Hepatic stellate cells retain retinoid-laden lipid droplets after cellular transdifferentiation into activated myofibroblasts. American Journal of Physiology-Gastrointestinal and Liver Physiology, 315(5):713–721 PP.
5. Karsdal, M. A., Hjuler, S. T.; et al (2019). *Assessment of liver fibrosis progression and regression by a serological collagen turnover profile.* American Journal of Physiology-Gastrointestinal and Liver Physiology, 316(1): 25–31 PP.
6. Lambrecht, J., Verhulst, Mannaerts; et.al, (2019). *A PDGFRβ-based score predicts significant liver fibrosis in patients with chronic alcohol abuse, NAFLD and viral liver disease*. EBioMedicine, 43: 501–512 PP.
7. Maren S. Fragala, Adam R. Jajtner, Kyle S. Beyer, Jeremy R. Townsend ; et al2014*Biomarkers of muscle quality: N-terminal propeptide of type III procollagen and C-terminal agrin fragment responses to resistance exercise training in older adults.* ORIGINAL ARTICLE . 5:139–148PP
8. Ramírez-Fernández, O. (2020). Fibrosis and Hepatic regeneration mecanism. Translational Gastroenterology and Hepatology,Mexico : 23 PP.
9. Shrivastava, S. R., & Shrivastava, P. S. (2019). *Global elimination of viral hepatitis: Challenges and encouraging factors.* Medical Journal of Babylon, 16(1): 82 PP.
10. Trivella, J. P., Martin, P., & Carrion, A. F. (2020). *Novel targeted therapies for the management of liver fibrosis.* Expert Opinion on Emerging Drugs, 25(1):59–70 PP.
11. Wangensteen, K. J., & Chang, K.-M. (2021). *Multiple roles for hepatitis B and C viruses and the host in the development of hepatocellular carcinoma.* Hepatology (Baltimore, Md.), 73(1): 27 PP.