ORIGINAL ARTICLE

Prediction of Liver Fibrosis in Patients with Chronic Hepatitis C by Biochemical Surrogate Markers

MUZZAFAR MEHDI[1], ALI HYDER MUGHERI[2], MAHESH KUMAR[3], ASIF RAZA ZAIDI[4], WALI KHAN[5], ALTAF ALAM[6]
[1]Senior Registrar, [4]Assistant Professor, [6]Ex-Professor & Head, Department of Gastroenterology, [5]Senior Registrar, Hepatobiliary Unit, Shaikh Zayed Hospital, Lahore
[2]Associate Professor, [3]Senior Registrar, Department of Gastroenterology, Shaheed Muhtarma Benazir Bhutto Medical University/Chandka Medical College Hospital, Larkana
Correspondence to Dr. Muzzafar Mehdi E-mail: muzzafarmehdi@yahoo.com Cell 0333-4119220

ABSTRACT

Aim: To determine the relative viability of Fibro Test with liver biopsy in location of level of necrosis and fibrosis in chronic hepatitis C patients.
Study Design: Cross-sectional observational study.
Place and Duration of Study: Department of Gastroenterology, Shaikh Zayed Hospital, Lahore from 1st April 2016 to 31st March 2017.
Methodology: One hundred cases that had chronic hepatitis C were included. Clotting profile and liver biopsy was completed in proper hygienic conditions and samples were delivered to find Condell recording. On the same day a 10 cc specimen of blood was taken in which 3 cc for ALT, GGT and total bilirubin and 7cc for Fibro Test. Immuneturb method (ROCHE) was analyzed for fibro test while Flex® reagent cartridge was completed for ALT, GGT and total bilirubin.
Results: The grades of fibrosis were found between F0 and F4. The average ages for all METAVIR status cases were 41±12 years. Pair-wise age comparison of stage F1 revealed that F2 and F3 were quite higher and significant while significant in various stages of alpha2 macroglobulin levels. Haptoglobin levels were found noteworthy with p-value 0.005 and GGT levels were found substantial with p-value <0.001. Total bilirubin levels were found noteworthy with p-value 0.003 for various groups, Apolipoprotein A1 levels were found significant with p-value <0.001 and fibro score levels for various stages with p-value <0.001. 95.6% accuracy was found for the prognosis of all stages.
Conclusion: Fibro test is a protected, reliable, advantageous and generally economical blood test that uses six blood serum markers to make a score that relates well with the level of hepatic fibrosis. Fibro Test has been broadly approved in patients with hepatitis C.
Keywords: Fibro Test, fibrosis grade, necrosis, chronic hepatitis C

INTRODUCTION

The administration of developing quantities of hepatitis C cases is a key clinical issue. As per World Health Organization (WHO), about 170 million people are tormented with Hepatitis C over the world. The circumstance in Pakistan is like other creating nations. These figures propose that around 10 million people experience the ill effects of HCV disease crosswise over Pakistan[1]. Prolonged HCV contamination by and large incorporates harm to liver and aggravation of the liver that evidently causes fibrogenesis. Amid the advancement of fibrosis, the amount of these constituents increment as well as experience changes in their synthesis and structure.[2] Biopsy is still the first blessing in chronic hepatitis under limitation and predictive correctness of biochemical indicators[3] so, it's necessary to get knowledge and then decide that patients should be treated or not for the progression of liver damage and treatment response[4].

Fibro test (biochemical pointers for fibrosis of the liver) is an obtrusive substitute for biopsy of the liver for delayed HCV cases[5]. Various investigations have proposed the prognostic importance of two mixes of straightforward serum biochemical markers, Fibro Test and HCV-Fibosure USA for the assessment of liver fibrosis[6]. The test utilizes a count to combine the biochemical marker results got from standard demonstrative research office tests, with the age and sex of the patients to give an empiric speculation of fibrosis of the liver connecting from 0 to 1, with scores >0.6 considered to address basic fibrosis. The essential standard of Fibro Test is to lessen the quantity of liver biopsies complete for valuation of liver fibrosis. Alpha2 macroglobulin, haptoglobin, γ-glutamyl transpeptidase, apolipoprotein A1, and absolute bilirubin are essential biomarker measures in fibro test which is exceptionally explicit to age and sex.[7]

The primary reason for study is to decide the relative viability of Fibro Test with liver biopsy in recognition of level of fibrosis and rot in patients with chronic hepatitis C.

Received on 23-11-2020
Accepted on 07-01-2021

MATERIALS AND METHODS

This cross-sectional study was conducted at Department of Gastroenterology, Shaikh Zayed Hospital Lahore from 1st April 2016 to 31st March 2017. 100 patients having positive HCV antibody in serum were included. Chronic liver disease, socio-demographic data, risk factors, clinical, biological, virological, radiological and histological profile was obtained through detailed questionnaire. Liver biopsy was completed in proper hygienic conditions and samples were delivered to find Condell recording. To avoid intra observer variability, samples were reviewed by senior histopathologists at Indus laboratories Shadman Lahore. The METAVIR score grades, the degree of fibrosis on a 5-point scale from 0 to 4 and the activity (intensity of necro-inflammatory lesions) is graded on a 4-point scale from A0 to A3 was obtained. ALT, GGT and total bilirubin was done on the day of sampling by enzymatic method in

Department of Biochemistry Shaikh Zayed Hospital, Lahore. ALT and GGT was done on Dimension® clinical chemistry system (Dade Boehring) by using Flex® reagent cartridge. While the total bilirubin was done on the same equipment by a method that is a modification of Doumas reference method. SPSS version 20 was used. P value ≤0.05 was statistically significant.

RESULTS

There were 39 cases of grade F1 were equal gender ratio. The average ages, all biochemical level and fibro score for all METAVIR status patients were noted and their comparison were found significant with p-value <0.001 (Table 1). Assessment of pair wise found that age of stage F2 and F4 were of significantly higher than stage F1. The differences between all other groups were found insignificant (Table 2).

Pair wise examination uncovered that the patients with stage F4 had fundamentally higher normal α2 macroglobulin level when contrasted with the patients with stage F0, F1 and F2 with all p-values <0.001. Additionally the F3 organize patients had fundamentally larger amounts as contrast with F0, F1 and F2. F2 had altogether higher α2 macroglobulin levels when contrasted with F0 and F1, though F1 had fundamentally higher α2 macroglobulin level as contrast with F0. Pair-wise correlation of stage F3 had altogether higher normal complete bilirubin level when contrasted with the patients with stage F0, F1 and F2. Also, pair-wise examination of patients with stage F2, F3 and F4 had fundamentally higher normal Apo A dimension when contrasted with the patients with stage F0 and F1 likewise the F3 and F4 arrange patients had altogether larger amounts when contrasted with F2. The contrasts between all were non-significant (Table 3).

Pair wise examination noted that the patients with stage F4 had essentially lower normal haptoglobin level when contrasted with the patients with stage F0, F1, comparatively F3 arrange patients had fundamentally lower normal haptoglobin levels when contrasted with F0 and F1. F2 had fundamentally lower haptoglobin levels when contrasted with F0 and F1. Pair-wise correlation uncovered that the patients with stage F4 had essentially higher normal GGT level when contrasted with the patients with stage F0, F1 and F2, comparatively the F3 organize patients had altogether larger amounts as contrast with F0 and F1 and F2. F2 had essentially higher GGT levels as contrast with F0 and F1. In addition, examination of patients with stage F1, F2, F3 and F4 had fundamentally higher normal fibro score level when contrasted with the patients with stage F0. Also the F2, F3 and F4 organize patients had essentially larger amounts when contrasted with F1 results were non-significant (Table 4).

At this phase when fibro score was determined further it was evident that the normal fibro score was clearly different between various stages. The underlying cut off was chosen to be 0.10 underneath which the affectability of fibro score to identify F0 was 100 % with a particularity of 98.8%. The second range utilized for F1 was 0.101– 0.305 with an affectability of 79.5 and particularity of 100%. The range 0.306– 0.475 was distinguishing F2 with 90.6% affectability and 89.7% explicitness. The range for F3 was somewhere in the range of 0.476 and 0.685 with 100% affectability and 96.9% particularity and the F4 organize was identified with 100% affectability and explicitness at an estimation of fibro score >0.685. 95.6% accuracy was found for the prognosis of all stages (Table 5).

Table 1: Distribution of biochemical marker levels by their METAVIR status

METAVIR (Mean±SD)	Age	Alpha2 macroglobulin levels	Haptoglobin level	GGT level	Total bilirubin level	Apolipoprotein A1 (Apo A)	Fibro score
F0	41±12	2.7±0.5	1.3±0.7	68±30	15±5	1.3±0.3	0.05±0.02
F1	35±10	3.1±0.3	1.1±0.7	68±24	15±4	1.4±0.3	0.25±0.05
F2	43±10	3.7±0.4	0.8±0.6	88±35	16±5	2.1±0.5	0.38±0.06
F3	46±13	4.5±0.6	0.3±0	140±48	23±3	3.1±0.3	0.56±0.06
F4	52±6	4.9±0.5	0.4±0	198±128	20±3	3.2±0.3	0.78±0.08
P-value	0.002	<0.001	0.005	0.000	0.002	0.000	0.000

Table 2: Pair-wise comparison of age of patients with different METAVIR status

(I) **METAVIR**	(J) **METAVIR**	**Mean difference (I-J)**	**Std. Error**	**P value**
F0	F1	5.664	2.905	0.299
	F2	-1.994	3.011	0.964
	F3	-4.400	5.786	0.941
	F4	-11.300	5.282	0.212
F1	F2	-7.658*	2.520	0.025
	F3	-10.064	5.546	0.371
	F4	-16.964*	5.018	0.009
F3	F3	-2.406	5.602	0.993
	F4	-9.306	5.080	0.362
F3	F4	-6.900	7.086	0.866

Table 3: Pair-wise comparison of alpha2 macroglobulin, total bilirubin and Apolipoprotein of patients with different METAVIR status

METAVIR (I)	METAVIR (J)	alpha2 macroglobulin		Total bilirubin levels		Apolipoprotein A1 levels	
		Mean difference (I-J)	P value	Mean difference (I-J)	P value	Mean difference (I-J)	P value
F0	F1	-.4364±0.1*	0.002	0.915±1.1	0.937	-0.056±0.1	0.987
	F2	-1.0137±0.1*	0	-0.147±1.2	1	-.7272±0.1*	0
	F3	-1.8240±0.2*	0	-7.080*±2.3	0.027	-1.7050±0.2*	0
	F4	-2.2350±0.2*	0	-4.388±2.1	0.254	-1.8070±0.2*	0
F1	F2	-.5773±0.09*	0	-1.062±1.0	0.838	-.6712±0.0*	0
	F3	-1.3876±0.2*	0	-7.995±2.2*	0.005	-1.6490±0.2*	0
	F4	-1.7986±0.1*	0	-5.303±2.0	0.079	-1.7510±0.1*	0
F2	F3	-.8103±0.2*	0.003	-6.933±2.2*	0.025	-.9778±0.2*	0
	F4	-1.2213±0.1*	0	-4.241±2.0	0.249	-1.0798±0.1*	0
F3	F4	-0.411±0.2	0.578	2.692±2.8	0.883	-0.102±0.2	0.996

*P<0.05 (Significant)

Table 4: Pair wise comparison of Haptoglobin, GGT and Fibro score levels among patients with different METAVIR status

METAVIR	No.	Haptoglobin levels			GGT levels			Fibro score		
		Mean rank	Sum of rank	P value	Mean rank	Sum of rank	P value	Mean rank	Sum of rank	P value
F0	20	33.5	670	0.261	27.95	559	0.511	10.95	219	
F1	39	28.21	1100		31.05	1211		39.77	1551	< 0.001
Total	59									
F0	20	32.23	644.5	0.03	19.95	399	0.014	10.5	210	
F2	32	22.92	733.5		30.59	979		36.5	1168	< 0.001
Total	52									
F0	20	14	280	0.019	10.8	216	0.008	10.5	210	
F3	4	5	20		21	84		22.5	90	0.002
Total	24									
F0	20	14.5	290	0.041	10.75	215	0.002	10.5	210	
F4	5	7	35		22	110		23	115	0.001
Total	25									
F1	39	40.72	1588	0.033	30.27	1180.5	0.01	21.18	826	
F2	32	30.25	968		42.98	1375.5		54.06	1730	< 0.001
Total	71									
F1	39	23.54	918	0.012	20.27	790.5	0.005	20	780	
F3	4	7	28		38.88	155.5		41.5	166	0.001
Total	43									
F1	39	23.95	934	0.036	20.14	785.5	0.001	20	780	
F4	5	11.2	56		40.9	204.5				< 0.001
Total	44									
F2	32	19.63	628	0.068	17.17	549.5	0.032	16.59	531	
F3	4	9.5	38		29.13	116.5		33.75	135	0.002
Total	36									
F2	32	19.44	622	0.532	17.08	546.5	0.006	16.5	528	
F4	5	16.2	81		31.3	156.5		35	175	< 0.001
Total	37									
F3	4	3.5	14	0.14	4.63	18.5	0.712	2.5	10	
F4	5	6.2	31		5.3	26.5		7	35	0.014
Total	9									

Table 5: Prognosis of fibro score against stages (ROC curve)

Value Cut off	METAVIR State	Sen	Spec.	AUC	95% CI	
≤ 0.1	F0	100	98.8	99.4	98.3	100.0
0.101-0.305	F1	79.5	100.0	89.7	82.0	97.5
0.306-0.475	F2	90.6	89.7	90.2	83.0	97.4
0.476-0.685	F3	100	96.9	98.4	96.1	100.0
> 0.685	F4	100	100	100.0	100	100.0

DISCUSSION

World Health Organization (WHO) appraises that, pretty much 170 million people might be contaminated with hepatitis C (HCV) around the world. Around, 10 million individuals in Pakistan are contaminated with HCV. Follow up studies spreading over more than 10 to 20 years propose that cirrhosis happens in up to 50% of constant hepatitis C patients, 185-187with an estimated danger of 10 % every year movement to cirrhosis. Unending contamination with HCV commonly results in damage and irritation of liver, which has all the earmarks of being in charge of the related fibrogenesis[8]. Measure of irritation and fibrosis uncovered on liver biopsy is the best clinical proof of ailment movement in unending HCV disease. Histological movement file (HAI: KNODELL score) and METAVIR score are the more much of the time utilized systems[9]. Most consideration has been centered on whether non-intrusive systems can identify the nearness or nonappearance of negligible (i.e., F0–F1), noteworthy (i.e., ≥F2), or progressed (i.e., ≥F3– F4) fibrosis as indicated by the METAVIR histological score[10,11]

Current investigation depends on legitimacy and exactness of Fibro Test in the determination of hepatic fibrosis. Results from each test are consolidated to decide gentle fibrosis (METAVIR F 0 to 1), huge fibrosis (METAVIR F2 to 4) or uncertain stage. Affectability and particularity for identification of critical fibrosis (F2 or more prominent) are around 75 and 85 percent, respectively[12].

In present examination, fibrosis grades found among patients were somewhere in the range of F0 and F4 with practically approach sexual orientation proportion. The discoveries proposed that sexual orientation alone isn't related with huge danger of fibrosis in incessant hepatitis C comparable discoveries were uncovered in another study[13].

Current examination found that p esteem mean the odds of higher evaluation of fibrosis in incessant hepatitis C with propelling age (Table 2). These discoveries are upheld by other studies[14]. The job of maturing in fibrosis movement could be identified with higher weakness to ecological variables, particularly oxidative worry, to decrease in blood stream, in mitochondria limit, or in safe capacities.[15] The impact of age on fibrosis and its movement is important to the point that it is difficult to evaluate any rate of fibrosis without considering the age at infection[16].

In the present investigation, arrange F4 had essentially higher normal alpha2 macroglobulin level when contrasted with the patients with stage F0, F1 and F2 with all p-values <0.001 (Table 1). Past investigation recorded that cleavage of alpha2macroglobulin may increment steadily with the advancement of fibrosis, which is a pointer to foresee liver fibrosis.[17] In current outcomes found that the normal haptoglobin level for F0 gather was 1.3±0.7 (Table 1) and in pair shrewd correlation uncovered that distinctions were all unimportant (Table 4). Thus, in past examination uncovered comparable discoveries. Anyway haptoglobin levels can prompt false negative impression as in a network based examination; the predominance of haptoglobin underneath the 99% percentile was not uncommon (4.7%)[18].

In current investigation, mean GGT level is found maximum in F4 organize (Table 1) though in Pair-wise correlation, contrasts were immaterial (table 4). Comparable investigation found no huge connection between the level of fibrosis and GGT levels in patients with perpetual hepatitis C. In any case, GGT levels were observed to be higher in patients

with a high movement score than in patients with a low action score, with a distinction moving toward measurable criticalness.[19] Correspondingly Apolipoprotein A1 (Apo A) level is found maximum in F4 arrange (Table 1) yet Pair-wise distinction were immaterial (Table 4.) These discoveries are as opposed to another examination who found no distinction in the dimension of mean plasma Apolipoprotein A1 among kids with and without serious fibrosis[20].

The fibro score level is present examination observed to be maximum in F4 organize (Table1) .Simultaneously, the distinction somewhere in the range of F3 and F4 was huge (Table 3). The exactness of the Fibro test has been evaluated in liver fibrosis because of unending hepatitis C in various investigations and in reality it is the most approved non-obtrusive test used to identify hepatic fibrosis[21,22].

In the present investigation, the general precision of fibro score, with interims given, for forecast of different stages was 95.6% (Table 5). Correspondingly, past examination found that Fibro Test might be less helpful for the identification of middle of the road phases of fibrosis (F2) contrasted and the extraordinary stages.[23] Another investigation exhibited high precision of the Fibro Test in diagnosing instances of steatohepatitis, with an AUC of 0.85 24. Past examination demonstrated a ROC of 0.69 and 0.91 for the analysis of critical fibrosis (F≥2) and liver cirrhosis.

CONCLUSION

Fibro Test is a protected, reliable, advantageous and generally economical blood test that uses six blood serum markers to make a score that relates well with the level of hepatic fibrosis. Fibro Test has been broadly approved in patients with hepatitis C.

REFERENCES

1.

1. Bhatti S, Manzoor S. Molecular epidemiology and clinical features of Hepatitis C Virus (HCV) in epidemic areas of Interior Sindh, Pakistan. Pak J Med Sci 2016; 32(5):1279.
2. Weiner FR, Czaja MJ, Zern MA. The use of molecular hybridization techniques as tools to evaluate hepatic fibrogenesis. Connective Tissue Health Dis 2017; 28: 107-30.
3. Mendes LC, Ferreira PA, Miotto N, Zanaga L, Gonçales E, Lazarini MS, et al. Transient elastography and APRI score: looking at false positives and false negatives. Diagnostic performance and association to fibrosis staging in chronic hepatitis C. Brazilian J Med Biol Res 2016;49(9).
4. Abdollahi M, Pouri A, Ghojazadeh M, Estakhri R, Somi M. Non-invasive serum fibrosis markers: A study in chronic hepatitis. Bioimpacts 2015;5(1):17.
5. Huang Y, Jeffrey GP, Adams LA. Hepascore and Its application to liver disease. Biomarkers Liver Dis 2016:1-24.
6. Everson GT, Helmke SM, inventors; University of Colorado Boulder, assignee. Method for assessment of hepatic function and portal blood flow. United States patent US 8,961,925. 2015 Feb 24.
7. Nano J, Ghanbari M, Wang W, de Vries PS, Dhana K, Muka T, et al. Epigenome-wide association study identifies methylation sites associated with liver enzymes and hepatic steatosis. Gastroenterology 2017; 153(4):1096-106.
8. Muller KR. The bystander effect in hepatitis C virus infection: cellular interactions between infected cells and uninfected cells (Doctoral dissertation).
9. Yilmaz B, Koklu S, Buyukbayram H, Yalçin K, Korkmaz U, Posul E, et al. Chronic hepatitis B associated with hepatic steatosis, insulin resistance, necroinflammation and fibrosis. Afr Health Sci 2015; 15(3):714-8.
10. Fraquelli M, Baccarin A, Casazza G, Conti CB, Giunta M, Massironi S, et al. Liver stiffness measurement reliability and main determinants of point shear-wave elastography in patients with chronic liver disease. Alimentary Pharmacol Therapeu 2016;44(4):356-65.
11. Lee J, Kim MY, Kang SH, Kim J, Uh Y, Yoon KJ, Kim HS. The gamma-glutamyl transferase to platelet ratio and the FIB-4 score are noninvasive markers to determine the severity of liver fibrosis in chronic hepatitis B infection. Br J Biomed Sci 2018;9: 1-7.
12. Curry MP, Nezam AH. Noninvasive assessment of hepatic fibrosis: Overview of serologic and radiographic tests. UpToDate, Waltham, MA: Walters Kluwer Health. 2016.
13. Fatovich L. Noninvasive methods, including transient elastography, for the detection of liver disease in adults with cystic fibrosis/Les méthodes non invasives, y compris l'élastographie transitoire, pour déceler les maladies hépatiques chez les adultes atteints de fibrose kystique. Canadian J Gastroenterol Hepatol 2015;29(3):139.
14. Roohi N, Mehjabeen SA. Effects of cigarette smoking on serum proteins profile in male active and passive smokers. Punjab Univ J Zool 2017;32(2):209-15.
15. Dholia N, Ramteke P, Varghese JF, Rani V, Yadav UC. Oxidative Stress-Induced Molecular and Genetic Mechanisms in Human Health and Diseases. In Free Radicals in Human Health and Disease 2015; 91-103.
16. Zeremski M, Dimova RB, Pillardy J, de Jong YP, Jacobson IM, Talal AH. Fibrosis progression in patients with chronic hepatitis C virus infection. J Infec Dis 2016;214(8):1164-70.
17. Gangadharan B, Zitzmann N, Dwek RA, inventors; University of Oxford, assignee. Clinical diagnosis of hepatic fibrosis using a novel panel of human serum protein biomarkers. United States patent US 9,012,162. 2015.
18. Poordad F, Schiff ER, Vierling JM, Landis C, Fontana RJ, Yang R, et al. Daclatasvir with sofosbuvir and ribavirin for hepatitis C virus infection with advanced cirrhosis or post-liver transplantation recurrence. Hepatology 2016;63(5):1493-505.
19. Eminler AT, Irak K, Ayyildiz T, Keskin K et al. The relation between liver histopathology and GGT levels in viral hepatitis: More important in hepatitis B. Turk J Gastroenterol 2014; 25: 411-5.
20. Fitzpatrick E, Dhawan A. Noninvasive biomarkers in non-alcoholic fatty liver disease: current status and a glimpse of the future. World journal of gastroenterology: WJG. 2014; 20(31):10851.
21. Fallatah HI. Noninvasive biomarkers of liver fibrosis: an overview. Advan Hepatol 2014; 2014.
22. Castera L. Non-invasive assessment of liver fibrosis in chronic hepatitis C. Hepatol Int 2011; 5(2):625–34.
23. Kayadibi H, Yasar B, Ozkara S, Serdar MA, Kurdas OO, Gonen C. The diagnostic accuracy of the Forns index, platelet count and AST to Platelet Ratio Index derived fibrosis index for the prediction of Hepatitis C virus-related significant liver fibrosis and cirrhosis. Scand J Clin Lab Invest 2014;74(3):240-7.
24. Poynard T, Lassailly G, Diaz E. Performance of biomarkers FibroTest, ActiTest, SteatoTest, and NashTest in patients with severe obesity: meta analysis of individual patient data. PLoS ONE 2012;7(3):
25. Kleiner DE, Chalasani NP, Lee WM, Fontana RJ, Bonkovsky HL, Watkins PB, et al. Hepatic histological findings in suspected drug-induced liver injury: systematic evaluation and clinical associations. Hepatology 2014; 59(2):661-70.