CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.3474157

Available online at: http://www.iajps.com ***Research Article***

STUDY TO ANALYZE THE USE OF NON-INVASIVE PARAMETERS TO DETERMINE VARIOUS STAGES OF LIVER FIBROSIS IN CHRONIC HEPATITIS C VIRUS (HCV) PATIENTS

Dr Zubia Farooq, Dr Rubab Ameer, Dr Maryem Tanweer
Islamic International Medical College, Rawalpindi

Article Received: August 2019 **Accepted:** September 2019 **Published:** October 2019

Abstract:
Objectives: *We aimed in this analysis to determine the different phases of liver fibrosis in chronic hepatitis C virus (HCV) patients by using non-invasive tool like Fibro-scan and bio-chemical parameters.*
Study Design: *This is a case control comparative type of study.*
Place and duration: *This analysis was conducted in Benazir Bhutto hospital Rawalpindi for the duration of one year from March 2018 to February 2019.*
Methodology: *Number of 759 patients were diagnosed by biochemical tests out of which 609 patients were chronic HCV and 150 were normal healthy controls. Number of 609 HCV cases were classified in 05 groups due to liver stiffness according to Fibro-Scan observations which enrolled patients with no fibrosis, mild fibrosis, moderate fibrosis, severe fibrosis and cirrhosis. Just such patients which expressed the existence of HCV RNA (PCR assay) in serum were enrolled in this analysis. HCV cases those were suffering from more than one infection with HIV and Hepatitis B virus were not involved in this analysis.*
Results: *Progressive stage disease cases with severe liver fibrosis and cirrhosis presented raised intensity of aspartate aminotransferase (AST), AST/ALT, gamma-glutamyl transferase (GGT), aminotransferase (ALT), total albumin, lower intensity of albumin, alkaline phosphate (ALP) and haptoglobins matched with controls and were observed significant on the basis of features where the value of P was less than 0.01. group of patients with cirrhosis present raised intensity of ALP, GGT, AST/ALT, AST, ALT and total bilirubin when matched with group of patients without liver complexities and were significant on the basis of facts where the value of P was less than 0.01. haptoglobin and albumin were substantially minimum where the value of P was less than 0.05 in cirrhotic cases versus those with no liver complexities*
Conclusion: *It is concluded through our analysis that mixture of usually accessible biochemical parameters and fibro-Scan are helpful to verify the liver fibrosis and cirrhosis in HCV patients.*
Key Words: *Fibrosis, Alkaline Phosphatase (ALP), Liver Cirrhosis, Aspartate Aminotransferase (AST), Hepatitis C virus (HCV), gamma- Glutamyl-transferase (GGT), Fibro-Scan, Biochemical Markers, Alanine Transaminase (ALT), Haptoglobins.*

Corresponding author:
Dr Zubia Farooq,
Islamic International Medical College,
Rawalpindi, Pakistan.

QR code

Please cite this article in press Zubia Farooq et al., ***Study to Analyze the Use of Non-Invasive Parameters to Determine Various Stages of Liver Fibrosis in Chronic Hepatitis C Virus (Hcv) Patients.,*** *Indo Am. J. P. Sci, 2019; 06(10).*

INTRODUCTION:

Major reason of chronic liver ailments is Hepatitis C virus (HCV) and is risk factor of life with substantial death rate. Chronic HCV is classified into swelling of liver and has a maximum variable clinical course directing to hepatocellular carcinoma and cirrhosis [1,2]. Randomly a number of 170 million people approximated to be affected by HCV. Conservative interaction to the blood and its product filthy with HCV is the major factor of spread of this ailment [3,4,5]. Maximum number of patients of severe HCV will ultimately develop chronically infested with obstinate high liver enzymes [6]. As many important coagulation influences, enzymes and protein are produced by liver so raised or reduced intensity of certain enzymes and protein direct liver damage. Indirect biochemical markers such as alanine aminotransferase (ALT), alkaline phosphatase (ALP), haptoglobin, albumin, serum bilirubin, gamma-glutamyl transferase (GGT) and aspartate aminotransferase (AST) are helpful to manage these patients. AST and ALT are supposed to be impartial liver specific enzymes. ALT is situated in cytoplasm whereas AST is located in both cytoplasm and mitochondria. Serum Intensities of ALT and AST are increased in liver ailments and maximum intensities direct to severe tissue damage. Any damage to bile duct or hindrance in bile duct outcome to be the raise of serum ALP function in flow of blood. GGT is a transpeptidase enzyme which is the more sensitive symptom of hepatobiliary ailment existed in bile duct and liver [7,8]. Any damage causing liver inflammation resulting in raised serum GGT levels. Patients which progress hepatocellular carcinoma have maximum intensity of bilirubin raises in liver ailments cholestasis [9]. Raised bilirubin intensity direct existence of chronic liver syndrome or its development to chronic phase because of HCV [10]. Albumin is a plasma developed by liver hepatocytes. Minimum intensity of albumin is an analyst of progressive liver ailment and cirrhosis. Tissues like kidney, skin and lungs almost develop haptoglobin alongside liver. Low level of haptoglobin signifies liver damage particularly when there is no anemia directing low progression of haptoglobin by liver [11]. An else noninvasive method is liver stiffness measurement and by the help of Transient Elastography scarring in liver could be predicted along efficiency and reliability. Maximum difficulty reproduces most progressive liver fibrosis [12,13,14]. Fibro-scan is comprised of a probe along vibrator and ultrasonic transducer. Minimum consistency vibration of slight amplitude moves from vibrator to the tissue. Elastic shear wave proliferation generated by vibration moves from the tissue and its velocity is straightly associated with the toughness of tissue. Shear wave proliferation from liver tissue is based on stiffness of tissue. Raised stiffness of tissue outcomes to be quicker shear wave proliferation. Raise in enzymes of liver generally take place when a person is diseased by HCV and stay maximum when patient get forward to chronic situation along complexities. So, it is vital getting knowledge about phases of liver fibrosis for remedy possibilities. Liver biopsy is just reliable procedure to analyze liver hardness. Though, biopsy holds few critical hazards to patient like death of patients, bleeding and pain, as an invasive method [18]. Maximum HCV patients which experience biopsy usually does not present any symptom of liver fibrosis or have just slight liver fibrosis because of the accessibility of investigative analyzations and knowledge of HCV [19].

So, conclusion of simple noninvasive methods of analyzing liver hardness are necessary to prevent patients experiencing needless liver biopsies. Few most significant examinations existing are alpha-2 macroglobulin and haptoglobin but they are not carried out regularly because of associated maximum price [20]. Hepatocellular wound can be analyzed with the help of noninvasive devices like simple biochemical markers and Fibro-scan. Amino transferases are some sensitive and significant biochemical markers of liver cell damage and their existence of maximum or minimum intensity in blood help identify liver damage. Existence of these biochemical markers shows acute or chronic condition of ailment in HCV cases. Complexities progress slightly over a long duration. The intensity of few of these biochemical methods vary on the basis of the phase of the chronic hepatitis C [21]. Anti HCV anti bodies were predicted in serum of HCV patients by the use of ELISA procedure which is 3rd generation ELISA [22,23]. Regularly accessible examinations with Fibro-scan observations are the emphasis of current analyses in evaluating different phases of liver hardness (Fibrosis) in chronic HCV cases. As none of the accessible noninvasive procedures alone are reactive but a mixture of these procedures can be carried out in analyzing chronic analyzation like accessible ways of noninvasive methods for analyzing phases of liver fibrosis. Liver enzymes like transpeptidases, Fibro-scan observation, physiological factors, haptoglobin, bilirubin, serum, albumin and aminotransferases in chronic HCV were used for this determination.

METHODOLOGY:

Number of 759 patients were included in this analysis where the number of 609 patients were chronic HCV

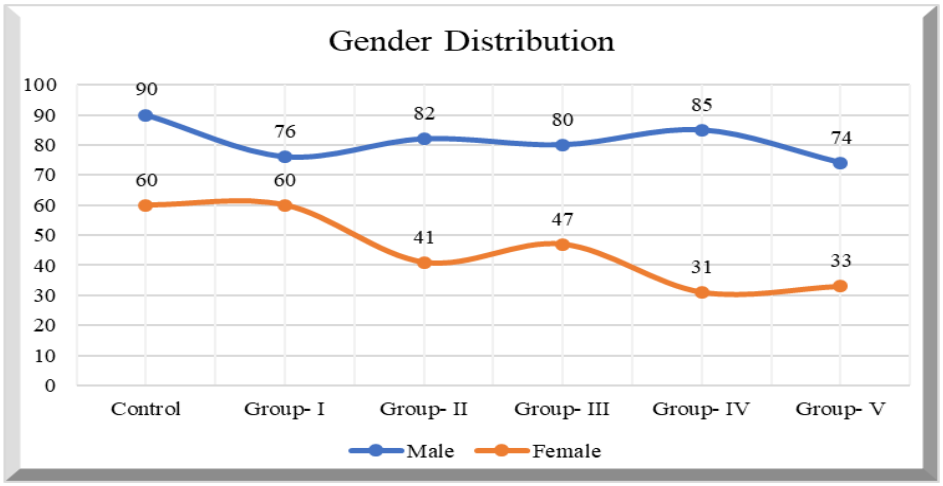
and 150 patients were normal healthy controls. These patients experienced Fibro-scan for the evaluation of hardness of liver. Patients were categorized in 5 groups on the basis of observations of Fibro-scan. Number of 136 patients with no liver fibrosis or no marking were included in Group I. number of 123 patients with slight fibrosis were included in Group II. Number of 127 patients having moderate fibrosis were positioned in the group III. Number of 116 patients with severe fibrosis were included in Group IV. Number of 107 patients with progressed cirrhosis were placed in group V. This analysis was comprised of just those HCV patients which presented presence of HCV RNA (PCR assay) in serum. HCV cases which were co-infected with HIV and Hepatitis B virus were not included in this analysis. This categorization was carried out on the basis of liver hardness calculation by the use of Fibro-scan. Data of these cases was noted on a predesigned form. A proper verification of the analysis was gotten from ethical and institution review board. For the biochemical study blood sampling with the value of 5ml were gathered from these patients and monitored in a gel tube. The blood samplings were left to clot for the duration of half an hour at the room temperature. These samples were centrifuged at 3000 rpm speed for ten minutes to obtain cell free clear serum after the completion of half an hour. Roche modular C501was processed to analyze ALP, albumin, total bilirubin, haptoglobin, ALT, GGT, AST. ALT/AST proportion was evaluated. Anti HCV anti bodies were predicted in serum of HCV patients by the use of ELISA procedure which is 3rd generation ELISA. SPSS 20 was carried out for the analyzation of data and total values were presented as mean ± SD. Student t-test and ANOVA methods were processed to perform matching of data. The cut value for consequence statistics was set at P less than 0.05.

RESULTS:

A mixture of biochemical outcomes and Fibro-scan were associated in the current analysis in different chronic HCV cases with histological complexities. Number of male and female patients out of 609 HCV patients was 397 and 212 with the percentage of 65.0 % and 35.0 % respectively stated in the patient group whereas control group consisting of male and female patients with the number of 90 and 60 with percentage of 60.0 % and 40.0 % respectively. Average age of control group patients was 39.39 ± 9.58 year. In patients of group I, II, III, IV and V the average age was noted as 41.6, 40.2, 46.5, 53.1 and 58.4 years accordingly. Age, gender and total number of patients included in this analysis are presented in the table no 01.

Table No 01: Age and Gender Distribution

Groups	Mean Age in years (Mean±SD)	Frequency	Male		Female	
			Quantity	%age	Quantity	%age
Control	38.39±9.58	150	90	60%	60	40%
Group- I	41.61±12.46	136	76	56%	60	44%
Group- II	40.20±13.17	123	82	67%	41	33%
Group- III	46.52±12.92	127	80	63%	47	37%
Group- IV	53.08±12.15	116	85	73%	31	27%
Group- V	58.38±9.57	107	74	69%	33	31%



Fibro-scan observations and statistical study of group I, II, III, IV, V and control group are presented in the table no 02. Control and patients group ALT/AST proportion matching is presented in table no 02. Average ALT intensities were under the normal limit whereas it was elevated in group I, II, III and group IV patients presenting a specific difference among patients' group and control group where the value of P was less than 0.01 as shown in table number 02.

Table No 02: Analysis of Variable Parameters and Fibro-scan Finding Reflecting Presence of Significant Fibrosis and Cirrhosis

Variable	Controls (n=150)	Group-I (n=136)	Group-II (n=123)	Group-III (n=127)	Group-IV (n=116)	Group-V (n=107)
ALT (U/L)	27.3±4.9	86.09±37.8^{+*}	104.9±35.2^{+*}	75.5±21.9^{+*}	63.62±22.4^{+*}	38.4±11.33
AST (U/L)	22.4±3.8	49.21±12.6^{+*}	71.3.0±22.2^{+*}	70.9±19.7^{+*}	91.96±23.6^{+*}	112.53±31.6^{+}
GGT (U/L)	25.76±5.0	31.74±8.6	47.35±12.39^{+*}	58.85±23.3^{+*}	110.31±23.6^{+*}	175.14±38.9^{+}
ALP (U/L)	188.78±75.5	207.49±53.6*	189.20±61.4*	195.71±51.5*	312.97±46.0*	388.50±72.2^{+}
AST/ALT	0.82±0.09	0.57±0.3*	0.68±0.3*	0.94±0.3*	1.44±0.8^{+*}	2.93±1.49^{+*}
T.Bili(mg/ld.)	0.73±0.1	0.80±0.3*	0.74±0.2*	0.80±0.3*	2.70±0.7^{+*}	4.15±1.25^{+}

ALT=Alanine Aminotransferase; AST=Aspartate Aminotransferase; GGT=Gamma-glutamyl transpeptidase; ALP=Alkaline Phosphatase; T. Bili=Total Bilirubin; ALB=Albumin; HAPTO=Haptoglobin

\+ Different from control significantly (P=0.01)

*Different from group-V (cirrhosis) significantly (P=0.05)

There was a specific difference in ALT intensity of group V where the value of P was less than 0.05 versus patients groups I, group II, Group III and Group IV. Group V ALT levels almost mildly raised but presents no irregularity. AST level in control group was normal whereas it was raised in all patient groups. The AST level raises as the patient progresses to most severe phase of the ailment. Group V patients presented raise in intensity of AST. AST level in all patient group as a matching to control group presented a specific difference where the value of P was less than 0.01. Matched to patient group V, AST intensity in group I, group II, group III and group IV presented a specific difference where the value of P was less than 0.05. GGT level in group I and control group cases were normal presenting a non-specific difference where the value of P was more than 0.05. GGT intensity in group II, III, IV and group V cases were raised versus control group presenting substantial difference where the value of P was less than 0.01.

Same as, GGT level in group I, group II, group III and group IV patients presented a substantial difference matched with group V patients where the value of P was less than 0.05. It was analyzed in this analysis that percentage of 86.0 % out of all HCV influenced patients have raised ALT values. Same as, percentage of 82.0 % had raised AST level and percentage of 71.0 % out of the patients presented a raised intensity of GGT. These are significant observation and with association of outcomes of Fibro-scan, it can be processed as analytic device in evaluating different phases of liver fibrosis in chronic HCV patients. Matching of ALT/AST is presented in the above table no 02. ALT/AST proportion in patients group I, II, III and control group remain less than 1 presenting indefinite difference where the value of P was more than 0.05. ALT/AST ratio raises progressively as patient develops to most complex phase of ailment and remain above than 1 in patient group IV and V.

ALT/AST ratio more than 1 is a strong sign of existence of severe liver fibrosis and cirrhosis. This proportion in patient group IV and group V was substantially variant from control group where the value of P was less than 0.01. ALP level in control and patient group I, II, III were normal presenting no specific difference where the value of P was above than 0.05. The ALP level in group IVand group V cases and control group presented a substantial difference where the value of P was less than 0.01. Overall bilirubin was in normal edge in control group, patient group I, group II, group III presenting un-substantial difference where the value of P was greater than 0.05. overall bilirubin was maximum in-patient group IV and group V presenting a specific difference where the value of P was less than 0.01. overall value of bilirubin in patient group V presented a substantial difference when matched with patient group I, II and group III where the value of P was less than 0.05. Patients out of group IV and group V had raised intensity of bilirubin with the percentage of 88.0 % and 91.0 % respectively. No substantial difference was recorded in albumin values of control and patient group I, group II and group III where the value of P was more than 0.05. A substantial reduction in intensity of albumin was recorded in patient group IV and group V versus control group where the value of P was less than 0.01. Same as, the albumin intensity of patients of group IV was instantly less versus patient group I, group II and group III where the value of P was less than 0.05. Haptoglobin was in the normal limit in control group and patient group I, II and III presenting no substantial difference where the value of P was more than 0.05 whereas instantly minimum in-patient group IV and group V versus control group where the value of P was less than 0.01.

Maximum difference in haptoglobin intensity was recorded in group V patient versus patient group I, group II and group III where the value of P was less than 0.05. minimum level of albumin and haptoglobin presents the existence of progressive grade of ailment. Patients of group IV and group V with the percentage of 83.0 % and 89.0 % had minimum intensity of albumin. Likewise, patient group IV and group V with the percentage of 83.0 % and 85.0 % presented minimum intensity of haptoglobin.

DISCUSSION:

The current analysis practice mixture of Fibro-scan observation and biochemical parameters as noninvasive method for the prediction of liver fibrosis and cirrhosis of patients with chronic HCV. ALT values were maximum in initial phase of the ailment with mild or no fibrosis that is signified as group I and group II patients amongst the aminotransferases. Serum bilirubin, ALP, GGT and AST intensities were raised showing liver complexities in the primary phase of the ailment signified by group IV and group V patients. As the patients go towards progressive phases of the ailment from group I to group IV, a regular raise was recorded in these limits excluding ALT. these outcomes match the analysis of Hyder M, et al whose outcomes presents that these liver parameters are maximum in viral hepatitis with the symptoms of fibrosis and cirrhosis [24]. Likewise, by our analysis, ALT intensities are maximum than AST in the beginning of the ailments whereas in progressive phase of liver fibrosis and cirrhosis, AST is maximum than ALT same as outcomes of Daniel PK, et al [25]. Raised level of AST than ALT was recorded in progressive phases of the ailment in our analysis which is like as the observations of earlier analyses. Sulkowski MS, et al. reported in his observations that use of ALT and AST are more valuable than biopsy in previous phases of the ailment [26]. Our observations recommend that these noninvasive biochemical parameters are more useful in previous phase of the ailment than invasive biopsy method and is likely to previous analyses [27]. The ratio of ALT/AST was raised in patients of our group IV and group V along severe fibrosis and cirrhosis. Williams AL, et al. stated likewise observations of raised ratio of ALT/AST in liver cirrhosis [28]. ALT/AST ratio persist less than 1.0 in our control group and 3 patient group that is group I, group II and group III and is more than 1.0 in our group IV and group V, patients with progressive cirrhosis and fibrosis. Same observation with raised AST/ALT ratio in liver cirrhosis and fibrosis are

observed in analysis done by Assay N, et al. and Inglesby TV, et al [29,30]. Mild raise in GGT intensity was existed in group II and group III patients whereas a raised level was observed in patients of group IV and group V. same observation was observed in the analysis of Luthfallah G, et al [31].

Group IV and group V cases with progressive liver cirrhosis and fibrosis in our analysis presented a raised intensity of alkaline phosphatase and serum bilirubin function same as Forns X, et al. analysis [32]. Our analysis presents that these simple parameters are high analyst of liver complexities and could be used with Fibro-scan observations for the determination of liver complexities in patients with chronic HCV. Same outcomes were earlier gotten through analysis of Pohl A, et al. and Bonacini M, et al [33,34]. Raised intensity of overall bilirubin and minimum level of albumin and haptoglobin in group IV and group V patients is an significant observation of our analysis. Our analysis focuses the significance of biochemical indicators and Fibro-scan. Same observations were stated by Poynard T, et al. presenting significance of Fibro-scan in non-availability of biopsy [35]. Patients with progressive phases of the ailment by our analysis have minimum level of haptoglobin. Same outcomes are gotten in an analysis started by Contreras RH, et al. who processed haptoglobin, total bilirubin, GGT and 3 else biochemical indicators for the prediction of substantial fibrosis [36]. Our observations are maintained by the work of Angulo P, et al. whose analysis presents the significance of low albumin, low platelet and AST/ALT proportion in progressed fibrosis in liver ailment [37]. Their work maintains our observation of minimum albumin and maximum overall bilirubin in patients with chronic HCV of group IV and group V.

CONCLUSION:

Our information cooperatively proposes that these biochemical parameters along with Fibro-scan are noninvasive and significant dynamic measure of liver cirrhosis and fibrosis. These parameters might be valuable in observing the risk of liver ailment development and decreasing raised number of biopsies in patients with chronic HCV on the other hand more sensitive markers of liver cirrhosis and fibrosis are required. It is concluded through our analysis that mixture of usually accessible biochemical parameters and fibro-Scan are helpful to verify the liver fibrosis and cirrhosis in HCV patients.

REFERENCES:

1. Afridi SQ, Ali MM, Awan F, Zahid MN, Afridi IQ, Afridi SQ, et al. Molecular epidemiology and viral load of HCV in different regions of Punjab, Pakistan. Virol J 2014; 11:24. DOI: 10.1186/1743-422X-11-24.
2. Afridi SQ, Zahid MN, Shabbir MZ, Hussain Z, Mukhtar N, Tipu MY, et al. Prevalence of HCV genotypes in district Mardan. Virology J 2013; 10:90. DOI: 10.1186/1743- 422X-10-90.
3. Brant LJ, Hurrelle M, Balogun MA, Klapper P, Ahmad F, Boxall E, et al. Sentinel laboratory surveillance of hepatitis C antibody testing in England: understanding the epidemiology of HCV infection. Epidemiol Infect 2007 Apr; 135(3): 417-26. DOI: 10.1017/ S095026880 6006832.
4. Khan MH, Farrell GC, Byth K, Lin R, Weltman M, George J, et al. Which patients with hepatitis C develop liver complications? Hepatology 2000; 31(2):513-20. DOI: 10.1002/ hep.510310236.
5. Kuo I, Hassan SU, Galai N, Thomas DL, Zafar T, Ahmed MA, et al. High seroprevalence and HIV drug use risk behaviors among injection drug users in Pakistan. Harm Reduct J 2006; 3:26. DOI: 10.1186/1477- 7517-3-26.
6. Bruce MG, Bruden D, McMahon BJ, Christensen C, Homan C, Sullivan D, et al. Hepatitis C Infection in Alaska Natives with persistently normal, persistently elevated or fluctuating alanine aminotransferase levels. Liver Int 2006; 26(6):643-9. DOI: 10.1111/j.1478-3231.2006. 01281.x.
7. Thomas L. Alanine aminotransferase (ALT), Aspartate aminotransferase (AST). Clinical Laboratory Diagnostics. 1 ed. 1999. TH-Books Verlag's gesellschaft Frankfurt. p. 55-65.
8. Moss DW, Henderson AR. Clinical enzymology. In: Clinical Chemistry. r d3 ed. 1999. WB Saunders Company, Philadelphia. p. 617-721.
9. Singhal A, Jayaraman M, Dhanasekaran DN, Kohli V. Molecular and serum markers in hepatocellular carcinoma: predictive tools for prognosis and recurrence. Crit Rev Oncol Hematol 2012; 82(2):116-40. DOI: 10.1016/j.critrevonc.2011.05.005.
10. Fattovich G, Giovanna G, Degos F, Tremolada F, Diodati G, Almasio P, et al. Morbidity and mortality in compensated cirrhosis type C: a retrospective follow-up study of 384 patients. Gastroenterology 1997; 112(2):463-72. DOI:10.1053/ gast.1997.v112.pm9024300
11. Trayhurn P, Wood IS. Adipokines: inflammation and the pleiotropic role of white adipose tissue. Br J Nutr 2004; 92(03):347-55. DOI: 10.1079/BJN20041213.
12. Boursier J, Vergniol J, Guillet A, Hiriart JB, Lannes A, Le-Bail B, et al. Diagnostic accuracy and prognostic significance of blood fibrosis tests

and liver stiffness measurement by Fibro Scan in non-alcoholic fatty liver disease. J Hepatol 2016; 65(3):570- 8. DOI: 10.1016/j.jhep.2016.04. 023.
13. Talwalkar JA, Kurtz DM, Schoenleber SJ, West CP, Montori VM. Ultrasound based treatment transient elastography for the detection of hepatic fibrosis; systemic review and meta-analysis. Clin Gastroenterol Hepatol 2007; 5(10):1214-20. DOI: 10.1016/ j.cgh.2007.07.020.
14. Fridrich-Rust M, Ong MF, Martens S, Sarrazin C, Bojunga J, Zeuzem S, et al. Performance of transient elastography for the staging of liver fibrosis: A meta-analysis. Gastroenterology 2008; 134(4): 960-74. DOI: 10.1053/j.gastro. 2008.01.034.
15. Saito H, Tada S, Nakamoto N, Kitamura K, Horikawa H, Kurita S, et al. Efficacy of non-invasive elastometry on staging of hepatic fibrosis. Hepatol Res 2004; 29 (2):97-103. DOI: 10.1016/j.hepres. 2004.03.007.
16. Ziol M, HandraLuca A, Kettaneh A, Christidis C, Mal F, Kazemi F, et al. Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with chronic hepatitis C. Hepatology 2005; 41(1):48-54. DOI: 10.1002/hep.20506.
17. Sandrin L, Tanter M, Gennisson J L, Catheline S, Fink M. Shear elasticity probe for soft tissues with 1-D transient elastography. IEEE Trans Ultrason Ferroelectr Freq Control 2002;49(4):436-46.
18. Vilar-Gomez E, Chalasani N. Non- invasive assessment of non- alcoholic fatty liver disease: Clinical prediction rules and blood-based biomarkers. J Hepatol 2018; 68(2): 305-15. DOI: 10.1016/j.jhep. 2017.11.013.
19. Forns X, Ampurdanes S, Sanchez- Tapias JM, Guilera M, Sans M, Sanchez-Fueyo A, et al. Long term follow up of chronic hepatitis C in patients diagnosed at a tertiary-care center. J Hepatol 2001; 35(2):265- 71. DOI: 10.1016/S0168-8278(01) 00088-5.
20. Oberti F, Valsesia E, Pilette C, Roussel MC, Bedossa P, Aube C, et al. Noninvasive diagnosis of hepatic fibrosis or cirrhosis. Gastroenterology 1997;113(5): 1609-16. DOI: 10.1053/gast.1997. v113.pm9352863.
21. Ni H, Soe HHKS, Htet A. Determinants of abnormal liver function tests in diabetes patients in Myanmar. Int J Diabetes Res 2012; 1(3):36-41. DOI: 10.5923/ j. diabetes.20120103.02.
22. Bates SM. D-Dimer Assays in Diagnosis and Management of Thrombotic and Bleeding Disorders. Semin Thromb Hemost 2012; 38(7):673-82. DOI: 10.1055/s-0032-1326782.
23. Spencer K, Price CP. Influence of reagent quality and reaction condition on the determination of serum albumin by bromocresol green dye-binding method. Ann Clin Biochem 1977; 14(2):105-15. DOI: 10.1177/0004563277 01400119.
24. Hyder M, Hasan M, Mohieldein AH. Comparative levels of ALT, AST, ALP and GGT in Liver associated diseases. Euro J Exp Bio 2013;3(2):280-84.
25. Daniel PK, Isselbacher KJ. Cirrhosis and its complications. In Fauci AS, Braunwald E, Isselbacher KJ, Wilson JD, Martin JB, Kasper DL, Hauser SL, Longo DL, editors. Harrison's th Principles of Internal Medicine. 14 ed. 1998. McGraw-Hill Medical Publishing Division, New York. p 1704-10.
26. Sulkowski MS, Mehta SH, Torbenson MS, Higgins Y, Brinkley C, de Oca RM, et al. Rapid fibrosis progression among HIV/hepatitis C virus-co-infected adults. AIDS 2007;21(16):2209-16. DOI: 10.1097/QAD.0b013e3282f10de9.
27. Okuda M, Li K, Beard MR, Showalter LA, Scholle F, Lemon SM, et al. Mitochondrial injury, oxidative stress, and anti-oxidant gene expressions are induced by hepatitis Cviruscoreprotein. Gastroenterology 2002; 122(2): 366-75. DOI:10.1053/gast. 2002.30983
28. Williams AL, Hoofnagle JH. Ratio of serum aspartate to alanine aminotransferase in chronic hepatitis. Relationship to cirrhosis. Gastroenterology 1988; 95(3):734- 9. DOI:10.1016/S0016-5085(88) 80022-2.
29. Assay N, Minuk GY. Serum aspartate but not alanine aminotransferase levels help to predict the histological features of chronic hepatitis c viral infections in adults. Am J Gastroenterol 2000; 95(6):1545-50. DOI: 10.1111/j.1572-0241.2000. 02027.x.
30. Inglesby TV, Rai R, Astemborski J, Gtuskin L, Nelson KE, Vlahov D, et al. A prospective, community-based evaluation of liver enzymes in individuals with hepatitis C after drug use. Hepatology 1999; 29(2): 590-6. DOI: 10.1002/hep. 510290 219.
31. Lutfullah G., Nazli R, Akhtar T. Serum Alanine Aminotransferase levels in Hepatitis C patients in Teaching Hospitals of Peshawar. J Chem Soc Pakistan 2008; 30(1):106- 9.
32. Forns X, Ampurdanes S, Llovet JM, Aponte J, Quinto L, Martinez-Bauer E, et al. Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. Hepatology 2002; 36(4 Pt 1):986-92. DOI: 10.1053/jhep. 2002.36128.
33. Pohl A, Behling C, Oliver D, Kilani M, Monson P, Hassanein T. Serum aminotransferase levels and platelet counts as predictors of degree of

fibrosis in chronic hepatitis C virus infection. Am J Gastroenterol 2001; 96(11):3142-6. DOI: 10.1111/j.1572-0241.2001.05268. x.

34. Bonacini M, Hadi G, Govindarajan S, Lindsay KL. Utility of a discriminant score for diagnosing advanced fibrosis or cirrhosis in patients with chronic Hepatitis C virus infection. Am J Gastroenterol 1997; 92(8): 1302-4.
35. Poynard T, de Ledinghen V, Zarski JP, Stanciu C, Munteanu M, Vergniol J, et al. Relative performances of Fibro Test, FibroScan, and biopsy for the assessment of the stage of liver fibrosis in patients with chronic hepatitis C: a step toward the truth in the absence of a gold standard. J Hepatol 2012;56(3):541-8. DOI: 10.1016/j.jhep.2011.08.007.
36. Contreras RH, Callewaert NLM. Serum marker for measuring liver fibrosis. US Patent 2007; 7244619.
37. Angulo P, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, et al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology 2007; 45(4): 846-54. DOI: 10.1002/hep.21496.