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NONINVASIVE PARAMETERS AND STAGING OF LIVER FIBROSIS IN CHRONIC HEPATITIS C PATIENTS

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ABSTRACT

OBJECTIVES: To use biochemical parameters and FibroScan as noninvasive tool in determination of various stages of liver fibrosis in chronic hepatitis C virus (HCV) patients.

METHODS: Total 759 participants (609 chronic HCV patients and 150 normal healthy controls) were recruited from Lady Reading hospital and Al-Hayat Medical Center, Dabgri garden, Peshawar, Pakistan and analyzed for biochemical markers from February 2015 to January 2017. On the basis of liver stiffness, 609 HCV patients were categorized in 05 groups as per FibroScan findings that include patients with no fibrosis, mild fibrosis, moderate fibrosis, severe fibrosis and cirrhosis. Only those HCV patients who showed presence of HCV RNA (PCR assay) in serum were included in this study. HCV patients who were co-infected with Hepatitis B virus and HIV were excluded from this study.

RESULTS: Advance staged disease patients with severe liver fibrosis and cirrhosis showed elevated level of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), AST/ALT, total bilirubin and lower level of albumin and haptoglobinas compared to controls and were found statistically significant ($P<0.01$). Patients group with cirrhosis showed elevated level of AST, ALT, GGT, ALP, AST/ALT and total bilirubin when compared to patients group with no liver complications and were statistically significant ($P<0.01$). Albumin and haptoglobin were significantly lower ($P<0.05$) in cirrhotic patients compared to those without liver complications.

CONCLUSION: Combination of FibroScan and routinely available biochemical parameters are helpful in identifying liver fibrosis and cirrhosis in chronic HCV patients.

KEY WORDS: Hepatitis C (MeSH); Fibrosis (MeSH); Liver Cirrhosis (MeSH); FibroScan (Non-MeSH); Biochemical Markers (MeSH); Aspartate Aminotransferase (MeSH); Alanine Transaminase (MeSH); gamma-Glutamyltransferase (MeSH); Haptoglobins (MeSH); Alkaline Phosphatase (MeSH).

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INTRODUCTION

Hepatitis C virus (HCV) is the leading cause of chronic liver diseases and is life-threatening with significant mortality rate. Chronic HCV is characterized by inflammation of liver and has a highly variable clinical course, leading to cirrhosis and hepatocellular carcinoma.[1,2] Roughly 170 million individuals around the world estimated to be the victims of HCV. Direct contact with the blood and its product contaminated with HCV is the main cause of transmission of this disease.[3-5] Majority of patients with acute HCV will eventually become chronically infected with persistent high liver enzymes.[6]

Since most of important proteins, enzymes and coagulation factors are synthesized by the liver, therefore increased or decreased level of certain protein and enzymes indicate liver damage. Indirect biochemical markers such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), serum bilirubin, albumin and haptoglobin are helpful to manage these patients. ALT and AST are considered important liver specific enzymes. ALT is located in cytoplasm, while AST is situated in both, mitochondria and cytoplasm. Serum ALT & AST levels are raised in liver diseases and high levels indicate severe tissue damage. Any damage to bile duct or obstruction in bile duct results in the increase of serum alkaline phosphatase activity in blood circulation. GGT, a transpeptidase enzyme is the most sensitive indicator of hepatobiliary disease present in liver and bile duct.[7,8] Any damage causing liver inflammation result in increased serum GGT levels. Patients who develop hepatocellular carcinoma have higher level of serum GGT. GGT and ALP both increases in obstructive liver disease and cholestasis.[9] Serum bilirubin level increases in liver diseases. Increased bilirubin level indicate presence of chronic liver condition or its

TABLE I: AGE AND GENDER DISTRIBUTION

Groups	Mean Age in years (mean ± SD)	Number of Individuals	Men	Women
			n (%)	n (%)
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)

progression to chronic stage due to HCV.[10] Albumin is a plasma protein produced by liver hepatocytes. Low albumin level is a predictor of cirrhosis and advanced liver disease. Tissues such as kidney, lungs and skin also produce haptoglobin alongside liver. Low haptoglobin level indicates liver damage particularly when there is no anemia indicating low haptoglobin production by liver.[11]

Liver stiffness measurement is another noninvasive procedure and with the help of Transient Elastography scarring in liver can be detected with great reliability and effectiveness. Higher stiffness reflects more advanced liver fibrosis.[12-14] FibroScan consist of a probe with ultrasonic transducer and a vibrator. Low frequency vibration of mild amplitude passes from vibrator to the tissue. Elastic shear wave propagation produced by vibration passes through the tissue and its velocity is directly related to the tissue stiffness. Shear wave propagation through liver tissue depend on tissue hardness. Increased tissue hardness results in faster shear wave propagation.[15-17]

Increase in liver enzymes usually occurs when an individual is infected with HCV and stay high when patients move towards chronic condition with complications. Therefore it is essential knowing stages of liver fibrosis for treatment options. Only reliable method in assessing liver stiffness is liver biopsy. However, as an invasive procedure, biopsy carries some serious risk to patient such as pain, bleeding and even death of patients.[18] Due to the availability of diagnostic tests and awareness about HCV, majority of HCV patients who undergo biopsy normally does not show any sign of liver fibrosis or have only mild liver fibosis.[19] Therefore, finding of simple noninvasive ways of assessing liver stiffness are required to avoid patients going through unnecessary liver biopsies. Some very specific tests available are haptoglobin and alpha-2 macroglobulin but they are not done routinely due to relatively high cost.[20]

Hepatocellular injury can be diagnosed with the help of noninvasive tools such as FibroScan and simple biochemical markers. Aminotransfereases are one of the important and sensitive biochemical markers of liver cell injury and their presence of high or low level in blood help recognize liver injury. Presence of these biochemical markers represents chronic or acute state of the disease in HCV patients. Complications develop slowly over a long period of time. The level of some of these biochemical parameters fluctuate depending on the stage of the chronic hepatitis C.[21] Stiffness in liver also predicts various stages of liver disease which can be detected with the help of fibroscan. Both liver stiffness and biochemical marker are important findings in HCV patients.[12,13]

Routinely available tests along with FibroScan findings are the focus of present study in differentiating various stages of liver stiffness (fibrosis) in chronic HCV patients. Since none of the available noninvasive methods alone are effective, a combination of these methods can be used in assessing chronic HCV patients. We aimed to use a combination of FibroScan finding and laboratory parameters assessment as available means of noninvasive approaches for assessing stages of liver fibrosis. Liver enzymes such aminotransferases, transpeptidases, serum albumin, bilirubin and haptoglobin, physiological factors and FibroScan finding in chronic HCV patients were utilized for this purpose.

METHODS

Total of 759 individuals (609 chronic

TABLE II: ANALYSIS OF VARIABLE PARAMETERS AND FIBROSCAN 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/dL)	0.73±0.1	0.80±0.3*	0.74±0.2*	0.80±0.3*	2.70±0.7$^{+,*}$	4.15±1.25^{+}
ALB (g/dL)	4.51±0.4	5.03±0.6*	4.97±0.8*	4.39±0.8*	3.42±0.55^{+}	2.32±0.60^{+}
HAPTO (g/L)	1.18±0.5	1.02±0.6*	0.99±0.6*	0.59±0.2*	0.21±0.07^{+}	0.12±0.06^{+}

ALT=Alanine Aminotransferase; AST=Aspartate Aminotransferase; GGT=Gamma-glutamyltranspeptidase;
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)

HCV patients and 150 normal healthy controls) participated in this study from February 2015 to January 2017. These individuals went through FibroScan for the determination of liver stiffness. Patients were classified into 5 groups based on FibroScan findings. Group-I consisted of 136 patients with no scarring or no fibrosis of liver. Group-II with mild fibrosis has 123 patients. Patients with moderate fibrosis were placed in Group-III with total number of 127 patients. Patients (116) with severe fibrosis were placed in Group-IV. Group-V consisted of 107 patients who developed cirrhosis. Only those HCV patients who showed presence of HCV RNA (PCR assay) in serum were included in this study. HCV patients who were co-infected with hepatitis B virus and HIV were excluded from this study. This classification was made based on liver stiffness measurement with the help of FibroScan. These patients visited Al-Hayat Medical Center Dabgri gardens Peshawar, Pakistan for FibroScan. Information from these patients was recorded on a proforma. A formal approval of the study was taken from ethical and institutional review board.

For the biochemical analysis blood samples (5 ml) were collected from patients and control in a gel tube. The blood samples were allowed to clot for 30 minutes at room temperature. At the end of 30 minutes, these samples were centrifuged at 3000 rpm speed for 10 minutes to get cell free clear serum. Roche modular C501 was used for the analysis of ALT, AST, GGT, ALP, albumin, total bilirubin and haptoglobin. AST/ALT ratio was calculated. Anti HCV anti bodies were determined in serum of HCV patients with the help of ELISA method (3rd generation ELISA).[22,23]

SPSS (version 16) was utilized for data analysis and all the values were expressed as mean ± SD. Data comparison was carried out with student t-test and ANOVA techniques. The cut of value for statistical significance was set at $P < 0.05$.

RESULTS

A combination of FibroScan findings and biochemical results were correlated in the present study in various chronic HCV patients with histological complications. Out of 609 HCV patients, 397 (65%) male and 212 (35%) female were recorded in patient group, while control comprising 90 (60%) male and 60 (40%) female volunteers. Mean age of controls was 38.39±9.58 years. In patients group-I, group-II, group-III, group-IV, group-V mean age recorded was 41.6, 40.2, 46.5, 53.1, 58.4 years respectively. Age, sex and total number of individuals participated in study are shown in Table I.

FibroScan findings and statistical analysis of control and group-I, group-II, group-III, group-IV and group-V patients are shown in Table II. Control and patients group AST/ALT ratio comparison are shown in Table II.

In control group, mean ALT levels were within the normal limit while it was elevated in group-I, group-II, group-III and group-IV patients showing a significant variation between patient groups and control ($P<0.01$) as shown in Table II. There was a significant variation ($P<0.05$) in ALT level of group-V as compared patients group-1, group-II, group-III and group-IV as shown in Table II. Group-V ALT levels although slightly elevated but shows no abnormality. AST level in control group was normal while it was elevated in all patient groups. The level of AST increases as the patient advances to more severe stage of the disease. Group-V patients showed highest elevation in AST level. AST level in all patient group as compared to control showed a significant variation ($P<0.01$) as shown in Table II. Compared to patient group-V, AST level in group-I, group-II, group-III and group-IV showed a significant variation ($P<0.05$). GGT level in control and group-I patients were normal showing a non-significant alteration ($P>0.05$). GGT level in group-II, group-III, group-IV and group-V patients were high compared to control showing a significant variation ($P<0.01$) as shown in Table II. Similarly, GGT level in group-I, group-II, group-III and group-IV patients showed a significantly variation compared to group-V patients ($P<0.05$). It was observed in this study that 86% of all HCV infected patients have elevated ALT values. Similarly, 82% of patients had elevated AST level and 71% of these patients showed an increased level of GGT. These are important finding and with correlation of FibroScan results, it can be used as diagnostic tool in differentiating various stages of liver fibrosis in chronic HCV patients.

Comparison of AST/ALT ratio is shown in Table II. AST/ALT ratio in patient group-I, group-II, group-III and control remain <1 showing a non-significant variation ($P>0.05$). AST/ALT ratio increases gradually as patient advances to more complicated stage of the disease and remain >1 in patient group-IV and group-V. AST/ALT ratio >1 is a strong indication of presence of severe liver fibrosis and cirrhosis. This ratio in patient group-IV and group-V was significantly different from control group ($P<0.01$).

ALP level in control and patient group-I, group-II, group-III were normal showing no significant variation ($P>0.05$). The ALP level in group-IV and group-V patients and control group showed a significant variation ($P<0.01$) as shown in Table II. ALP level in group-V patients showed a significant variation compared to patient group-I, group-II and group-III ($P<0.05$).

Total bilirubin was within normal limit in control group, patient group-I, group-II, group-III showing no significant variation ($P>0.05$). Total bilirubin was high in patient group-IV and group-V showing a significant variation ($P<0.01$) as shown in Table II. Total bilirubin value in patient group-V showed a significant variation when compared to patient group-I, group-II and group-III ($P<0.05$). From group-IV 88% and 91 % of group-V patient had elevated level of bilirubin. No significant variation was noted in albumin values of control and patient group-I, group-II and group-III ($P>0.05$). A significant decrease in albumin level was noted in patient groups-IV and group-V compared to control ($P<0.01$) as shown in Table II. Similarly, the albumin level of group-V patients were significantly lower compared to patient group-I, group-II and group-III ($P<0.05$).

Haptoglobin was within normal limit in control and patient group-I, group-II and group-III showing no significant

variation ($P>0.05$) while significantly lower in patient groups-IV and group-V compared to control ($P<0.01$) as shown in Table II. High variation in haptoglobin level was noted in group-V patient compared to patient group-I, group-II and group-III ($P<0.05$). Lower level of albumin and haptoglobin shows the presence of advanced degree of disease. 83% of group-IV and 89% of group-V patients had lower level of albumin. Similarly, 79% of group-IV and 85 % of group-V patient showed lower level of haptoglobin .

DISCUSSION

The present study use combination of biochemical parameters and FibroScan finding as noninvasive tool for the determination of liver fibrosis and cirrhosis in chronic HCV patients.

Among the aminotransferases, ALT values were higher in early stage of the disease with lesser or no fibrosis which is represented by group-I and group-II patients. In advanced stage of the disease represented by group-IV and group-V patients, serum bilirubin, GGT, AST and ALP levels were increased indicating liver complications. A gradual increase was noted in these parameters as the patient moves to advance stages of the disease (from group-I to group-IV) with the exception of ALT. These results resemble the work of Hyder M, et al.[24] whose results shows that these liver parameters are higher in viral hepatitis with signs of fibrosis and cirrhosis. Similarly, in our study ALT levels are higher than AST in start of the disease while in advance stages of liver fibrosis and cirrhosis, AST is higher than ALT similar to the results of Daniel PK, et al.[25] Increased level of AST than ALT was noted in advance stages of the disease in our study which is similar to the findings of previous studies. Sulkowski MS, et al.[26] reported in his finding that use of AST and ALT are more important than biopsy in early stages of the disease. Our findings suggest that these noninvasive biochemical parameter are more beneficial in early stage of the disease than invasive biopsy procedure and is similar to previous studies.[26,27] Patients in our group-IV and group-V with severe fibrosis and cirrhosis have increased AST/ALT ratio. Similar findings of increased AST/ALT ratio in liver cirrhosis are reported by Williams AL, et al.[28] AST/ALT ratio remains <1.0 in our controls and three patients group i.e. group-I, group-II and group-III and is >1.0 in our group-IV and group-V, patients with advance fibrosis and cirrhosis. Similar finding with increased AST/ALT ratio in liver fibrosis and cirrhosis are found in study by Assay N, et al. [29] and Inglesby TV, et al.[30]

A slight increase in GGT level was present in group-II and group-III patients while an increased level was found in patients of group-IV and group-V. Similar finding was found in the study of Luthfullah G, et al.[31]

Group-IV and group-V patients with advance liver fibrosis and cirrhosis in our study showed an increased level of alkaline phosphatase and serum bilirubin activity similar to Forns X, et al.[32] study. Our study shows that these simple parameters are strong predictor of liver complications and can be utilized along with FibroScan findings for the prediction of liver complications in chronic HCV patients. Similar results were previously obtained by study of Pohl A, et al.[33] and Bonacini M, et al.[34]

Increased level of total bilirubin and low level of albumin and haptoglobin in group-IV and group-V patients is an important finding of our study. Our study highlights the importance of biochemical markers and FibroScan. Similar finding are reported by Poynard T, et al.[35] showing importance of biochemical markers and FibroScan in the absence of biopsy. Patients with advanced stages of the disease in our study have low level of haptoglobin. Similar results are obtained in a study conducted by Contreras RH, et al.[36] who used haptoglobin, GGT, total bilirubin and three other biochemical markers for the determination of significant fibrosis.

Our findings are supported by the work of Angulo P, et al.[37] whose study shows the importance of low platelet, low albumin and AST/ALT ratio in advance fibrosis in liver diasease.[37] Their work support our finding of low albumin and high total bilirubin in chronic HCV patients of group-IV and group-V.

CONCLUSION

Collectively our data suggest that these biochemical parameters together with FibroScan are noninvasive and important dynamic measure of liver fibrosis and cirrhosis. These parameters may be beneficial in assessing the risk of liver disease progression and reducing increased number of biopsies in chronic HCV patients nonetheless more sensitive markers of liver fibrosis and cirrhosis are needed.

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AUTHORS' CONTRIBUTIONS

Following authors have made substantial contributions to the manuscript as under:

AR: Acquisition of data, drafting the manuscript, final approval of the version to be published.

TS: Acquisition of data, final approval of the version to be published.

RN & GL: Concept & study design, drafting the manuscript, critical review, final approval of the version to be published.

SF & AZ: Analysis & interpretation of data, drafting the manuscript, final approval of the version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

CONFLICT OF INTEREST

Authors declared no conflict of interest

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